CHANGES IN PLASMA INORGANIC PHOSPHATE ASSOCIATED WITH ENDOCRINE ACTIVITY IN XENOPUS LAEVIS.

S U M M A R Y of T H E S I S

Presented for the degree of

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(by)

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SUMMARY OF THESIS.

INTRODUCTION

The plasma inorganic phosphate level may be influenced by

(1) factors affecting the intermediary metabolism of phosphorus,

(2) alteration in the degree of absorption of phosphorus,

(3) alteration in the degree of excretion of phosphorus.

In the introduction, the intermediary metabolism of phosphorus, particularly as far as the endocrine glands are concerned, is discussed in detail, whereas the absorption and excretion are but briefly outlined.

(A) FACTORS INFLUENCING THE LEVEL AND STATE OF PHOSPHORUS IN THE BLOOD STREAM.

State of phosphorus in the blood stream. One inorganic and three organic compounds are found in the blood. Little is known about the intermediary metabolism of the organic fraction.

The following factors, Species difference, Age, Seasonal variations may each affect phosphorus metabolism. Calcium metabolism is closely associated with phosphorus metabolism. Serum calcium and blood inorganic phosphate are inversely related to each other.

Phosphorus plays an important part in the regulation of the acid-base equilibrium.

Phosphorus .....

Phosphorus is required for the metabolism of carbohydrates, fats and proteins.

The Parathyroid Glands.

(1) Parathyroidectomy leads to a rise in blood inorganic phosphate and a fall in serum calcium. A fall in phosphorus excretion is characteristic of this condition.

(2) A single injection of parathyroid hormone into man and dogs leads to a fall in blood inorganic phosphate and a rise in serum calcium. There is an immediate outpouring of phosphorus in the urine. Other species e.g. cats, rabbits etc. are more resistent to the hormone.

Repeated injections of parathyroid extract leads to a striking hypercalcaemia and hyperphosphataemia. Massive doses are lethal and the terminal hyperphosphatemia may be extreme. The volume of urine and the phosphate excretion are significantly increased.

(3) Two theories for the mode of action of parathyroid hormone have been suggested. The hormone may act by mobilising calcium from the bones i.e. a primary effect on calcium metabolism, or the primary action may be on the kidney by lowering the renal threshold to phosphorus. The latter hypothesis has recently been receiving much support.

Vitamin D.

Vitamin D is intimately concerned in the metabolism of calcium and of phosphorus. It may be involved in the

absorption .....

absorption, excretion and intermediary metabolism of these elements. Recent work tends to show that it does not affect the absorption and excretion of phosphorus as it does that of calcium.

The Thyroid Gland.

(1) The thyroid gland affects the general metabolism of an organism and the mineral metabolism in particular.
By stimulating the oxidation of carbohydrates, it may affect phosphorus metabolism as phosphorus is required for the oxidation of glucose.

(2) Thyroidectomy leads to a slight decrease of serum calcium or no effect. No change in inorganic phosphate has been observed, but the literature is scanty.

(3) Administration of thyroxine leads to a slight increase in serum calcium. A slight decrease in inorganic phosphate has been recorded.

(4) Excretory changes are well known. Thyroxine mobilises calcium and phosphorus from the bones and an increased excretion of these elements occurs.

The Gonads.

(1) Gonadectomy leads to no change or a slight decrease of serum calcium. A slight fall of inorganic phosphate has been described.

(2) Injection of ovarian extracts have little effect on the

serum .....

serum calcium or inorganic phosphate.

(3) Changes in blood calcium during the menstrual cycle have been described. During pregnancy a fall in blood inorganic phosphate and in serum calcium have frequently been observed.

(4) In aves and amphibia a definite rise in serum calcium has been observed. This increase occurs before ovulation and increased parathyroid activity has been suggested to account for this phenomenon.

The Pancreas.

(1) Pancreatectomy produces a rise or no change in plasma inorganic phosphate. An increased elimination of phosphate occurs after pancreatectomy.

(2) Injection of insulin leads to a fall of plasma inorganic phosphate. A secondary hyperphosphataemia has also been observed.

(3) Ingestion of glucose also produces a fall in plasma inorganic phosphate and it has been suggested that this fall is produced by a reflex secretion of insulin. Phosphorus is required for the intermediary metabolism of glucose.

(4) The decreased blood inorganic phosphate is accounted for by an increased formation of organic phosphate compounds and not by an increased phosphate excretion. The excretion of phosphorus is diminished after insulin injection or glucose ingestion.

(5) An increase in serum calcium after insulin injection has

frequently .....

frequently been reported.

The Adrenal Gland.

(1) Adrenalectomy leads to a high blood inorganic phosphate level. This is probably a result of adrenal cortical not medullary deficiency.

(2) Injection of adrenaline leads to a fall in blood inorganic phosphate which is accounted for by an increased formation of hexose-phosphate at the expense of the inorganic phosphate. Concomitant with the fall in blood inorganic phosphate, there is a diminution of phosphate excretion.

A secondary hyperphosphataemic has been described. This has been ascribed to changes in the acid-base equilibrium.

(3) Adrenal cortex is known to affect carbohydrate metabolism and the various phosphorylating mechanisms. It may thus affect the blood inorganic phosphate.

(4) Changes in serum calcium after adrenaline injection have been described.

The Pituitary Gland.

A. The anterior lobe

The following possible mechanisms whereby the anterior pituitary may affect the blood inorganic phosphate have been discussed.

(1) An effect on the plasma constituents. Hypophysectomy leads to a fall in blood inorganic phosphate, serum calcium

remains .....

remains unaffected or shows a drop. Injection of anterior pituitary extracts produce a rise in inorganic phosphate and usually a rise in serum calcium.

(2) An indirect effect following changes in carbohydrate metabolism (possibly following changes in fat and protein metabolism). It is well known that the anterior pituitary affects the metabolism of carbohydrates, fat and protein profoundly. Phosphorus is required for the intermediary metabolism of these substances and a secondary effect on the blood inorganic phosphate may thereby be effected.

(3) An indirect effect following stimulation of some other gland or glands. The parathyroid glands, the gonads, the thyroid gland, the pancreas and the adrenal glands are all affected by secretions from the anterior lobe of the pituitary. These glands each have some effect on phosphorus metabolism. The anterior pituitary may affect the blood inorganic phosphatelevel through one or more of these glands.

(4) A direct effect on the renal threshold for phosphate. Hypophysectomy may lead to a decrease in the excretion of phosphorus. This may be due to a rise in the renal threshold for phosphate and consequently the anterior pituitary may normally depress the renal threshold for phosphate.

B. <u>The posterior lobe</u>

The posterior lobe may be involved in the metabolism of

carbohydrate ....

carbohydrate, fat and protein and consequently may affect the blood inorganic phosphate. Posterior lobe extracts protect animals against insulin hypoglycaemia.

(B) FACTORS INFLUENCING THE ABSORPTION OF PHOSPHORUS FROM THE ALIMENTARY TRACT.

Most phosphorus compounds are readily soluble in water or in fat. Many are miscible with both. Organic compounds are hydrolysed and the inorganic phosphate finally released is readily absorbed. Phosphorus is very important in the absorption of calcium. Insoluble calcium phosphate salts may be formed by an excess of phosphates. The hydrogen ion concentration is of utmost importance in the absorption of calcium.

Vitamin D increase the amount of calcium absorbed but it is doubtful if it affects the absorption of phosphorus. Experiments with radio phosphorus in rats show that about 60 per cent of the ingested phosphorus is absorbed in 6 hours. Vitamin D is not required for the absorption of radio-phosphorus.

(C) FACTORS INFLUENCING THE EXCRETION OF PHOSPHORUS.

Phosphorus is mainly excreted by the kidneys. Some phosphorus is secreted in the intestinal juices and is subsequently excreted. The excretion of phosphate is largely regulated in such a way as to maintain the acid-base

equilibrium ....

equilibrium of the blood.

The ductless glands have an important influence on the excretion of phosphate as already described.

The nature of the diet especially the relative amounts of calcium and phosphorus have an influence on the excretion.

Vitamin D may possibly effect the excretion of calcium and phosphorus.

EXPERIMENTAL.

The following experiments were performed:-

(1) The effect of temperature changes from 5°C to 23°C on the level of the plasma inorganic phosphate of the South African clawed toad, Xenopus laevis.

(2) Seasonal changes in plasma inorganic phosphate of male and female toads.

(3) The effect of captivity on the plasma inorganic phosphate of normal female animals.

(4) The effect of hypophysectomy, of gonadectomy, of thyroidectomy and of pancreatectomy on the plasma inorganic phosphate.

(5) The effect of injection of acid extracts of the anterior pituitary, of parathyroid hormone, of oestradiol benzoate, of progesterone, of thyroxine sodium, of insulin and of

adrenaline .....

adrenaline on the plasma inorganic phosphate of normal animals.

(6) The effect of injection of acid extracts of the anterior pituitary of parathyroid hormone, of oestradiol benzoate, of progesterone, of thyroxine sodium, of insulin and of adrenaline on the plasma inorganic phosphate of anterior lobe hypophysectomised animals.

(7) The effect of repeated injections of parathyroid hormone, and of thyroxine sodium on the plasma inorganic phosphate of normal animals.

(8) The effect of insulin injection on pancreatectomised animals.

(9) The effect of injection of acid extracts of the anterior pituitary on castrated animals

General Discussion...

GENERAL DISCUSSION.

(1) The objects of this investigation have been outlined.

(2) A description of the following has been given:-

a. The method of collection and the storage of the animals.

b. The collection of blood, the use of anticoagulants and the preparation of heparin.

c. The biochemical methods of estimation of plasma inorganic phosphate used in this investigation.

d. The method of anaesthesia and the post-operative care employed.

(3) Hypophysectomy was performed according to the method of Hogben (1923), thyroidectomy according to that of Gitlin (1939a) and gonadectomy according to that of Shapiro (1933b). Pancreatectomy has been described for the first time.

(4) The method of injection, the preparation of anterior pituitary extract and the hormones used in this investigation have been described.

(5) Experiments are described to show that a range of 15°C does not affect the level of plasmainorganic phosphate of the South African clawed toad. (Xenopus laevis)

(6) Whereas the serum calcium level is higher in females than in males throughout the normal reproductive cycle, the

plasma .....

plasma inorganic phosphate is higher in females only during the pro-oestrous and anoestrous periods.

(7) The reproductive cycle in females is associated with changes in serum calcium and plasma inorganic phosphate levels and in ovarian activity. In male toads the plasma inorganic phosphate falls slightly during the breeding season just as in female toads, but as this fall is not marked, the plasma inorganic phosphate of male and of female toads are the same during the breeding season. The serum calcium of male toads, in contrast, remains remarkably steady.

(8) Captivity first causes a fall, then a steadily maintained elevated plasma inorganic phosphate. Serum calcium first rises then steadily declines in female toads.

(9) It is suggested that seasonal variations in the amount of solar radiation and inadequate illumination during captivity may be important agencies influencing the plasma inorganic phosphate and the serum calcium through the parathyroid glands and the pituitary; and perhaps ovarian activity through the anterior lobe of the hypophysis.

(10) Changes occurring due to seasonal variations and captivity involve several endocrine glands, particularly the pituitary, the parathyroids and the gonads and necessitate investigation of the relation of these glands to plasma inorganic phosphate.

(11) Removal of the anterior lobe of the pituitary (i.e.

para .....

pars anterior and pars tuberalis) leads to a significantly lower plasma inorganic phosphate level than in operative controls.

(12) When the pars tuberalis regenerates the plasma inorganic phosphate remains low in spite of the return of serum calcium to the normal level.

(13) The progressive fall in plasmainorganic phosphate is due to the removal of the anterior lobe of the pituitary alone.

(14) The fall in plasma inorganic phosphatemay be observed within one week of hypophysectomy and is fairly well marked within 2 to 3 weeks.

(15) Injection of anterior pituitary extract into anterior lobe hypophysectomised animals produces a rise of plasma inorganic phosphate to the preoperative level then a fall below the pre-injection level.

(16) Injection of anterior pituitary extract into normal animals produces an initial rise in plasma inorganic phosphate followed by a fall below the pre-injection level.

(17) The anterior pituitary affects calcium and inorganic phosphate in the same direction and it is probable that these two substances are affected through different mechanisms. The fall in serum calcium following hypophysectomy may be due to hypoparathyroidism but this cannot explain the fall in plasma inorganic phosphate.

(18) .....

(18) The possible modes of action of the anterior lobe of the pituitary are;

a. by altering the plasma constituents,

b. by affecting carbohydrate and consequently phosphorus metabolism,

c. by stimulating some other gland e.g. the adrenal cortex, the thyroid, the parathyroids, the pancreas or the gonads.

d. By raising the renal threshold to phosphate (this would produce a fall of plasma inorganic phosphate on hypophysectomy and opposite effects with anterior pituitary extract injection).

(19) The secondary fall of plasma inorganic phosphate below the pre-injection level after injection of anterior pituitary extract may be due to a reflex stimulation of insulin secretion, by the hyperglycaemia, or direct stimulation of some other endocrine gland such as the thyroid.

(20) Changes occurring due to hypophysectomy and anterior pituitary extract injection may involve the other endocrine organs and necessitate investigation of the relation of these glands to plasma inorganic phosphate.

(21) Injection of potassium phosphate in amounts equal to the total blood inorganic phosphate content of a toad produces no effect on the plasma inorganic phosphate whereas injection of 0.5 ml. fluid produces a slight diluting effect on the plasma.

(22) .....

(22) Intraperitoneal or intralymphatic injection of parathyroid extract into normal females produces an initial fall then a rise in plasma inorganic phosphate above the pre-injection level.

(23) Repeated injections of parathyroid extract produce an elevated plasma inorganic phosphate level which returns to normal 48 hours after the cessation of injections.

(24) The normal physiological action of the parathyroids is probably to produce a fall in plasma inorganic phosphate. The subsequent rise is probably an overdosage phenomenon.

(25) Seasonal variations and captivity effects have been ascribed to changes in parathyroid activity. Results of experiments on the effect of parathyroid extract on plasma inorganic phosphate are in keeping with this hypothesis.

(26) The secondary fall in plasma inorganic phosphate consequent on injection of anterior pituitary extract may possibly be due to stimulation of the parathyroid glands by the extract with the discharge of parathyroid hormone into the blood-stream.

(27) No further light has been thrown on the actual mode of action of parathyroid extract.

(28) Gonadectomy produces no change in plasma inorganic phosphate 6 months (females) and 3 months (males) after operation. Serum calcium, however, shows a fall 2 weeks after ovariectomy

and .....

and 6 months after orchidectomy.

(29) It is probable that the change in calcium is affected partly by the anterior pituitary. Serum calcium and plasma inorganic phosphate are probably affected by different anterior pituitary principles.

(30) Seasonal variations in plasma inorganic phosphate are probably not due to ovarian activity.

(31) Injection of oestradiol benzoate and of progesterone produces a fall in plasma inorganic phosphate. Ovarian extracts produce a rise in serum calcium, but the responsible factor is probably not oestrin or progesterone.

(32) It is probable that the ovary in the amphibian has no effect on the plasmainorganic phosphate though it may affect the serum calcium either directly or indirectly.

(33) It is probable that the effect of mammalian extract on plasma inorganic phosphate is not strictly comparable with the effect of the animal's own ovaries on phosphorus metabolism. The amphibian ovary probably produces hormones which are not identical with the mammalian hormones, oestradiol and progesterone.

(34) Thyroidectomy produces no change in plasma inorganic phosphate up to 7 months after thyroidectomy.

(35) Injection of 0.1 mg. thyroxine produces a fall of plasmainorganic phosphate in normal animals with a subsequent return to the normal level.

(36) Repeated injection of thyroxine (total dose of 0.4 - 0.6mg.)

produces .....

produces an elevated plasma inorganic phosphate level which does not return to the normal level within 48 hours after cessation of injections.

(37) Under physiological conditions, the thyroid probably produces no change in plasma inorganic phosphate.

(38) The fall in plasma inorganic phosphate following injection of thyroxine is attributed to an increased demand for inorganic phosphate, consequent on increased metabolic activity.

(39) The rise in plasma inorganic phosphate following repeated injections of thyroxine is attributed to consumption of carbohydrate stores with consequent diminished demand for inorganic phosphate. Mobilisation of calcium and phosphate from the bones and the soft tissues probably assists this reaction.

(40) The secondary fall of plasma inorganic phosphate consequent on injection of anterior pituitary extract may possibly be due to stimulation of the thyroid gland with the discharge of thyroid hormone into the blood stream.

(41) Ether has no effect on plasma inorganic phosphate 6 hours after anaesthesia.

(42) Pancreatectomy produces a rise in plasma inorganic phosphate which appears within 3 hours after operation.

(43) Injection of insulin into pancreatectomised animals produces a reduction of plasma inorganic phosphate towards

the .....

the normal level.

(44) Injection of insulin into normal animals produces a fall in plasma inorganic phosphate with a rise above the pre-injection level when the animals show hypoglycaemic symptoms.

(45) A direct relationship between the pancreas and phosphorus metabolism is suggested.

(46) The effect of insulin is a dual one namely;

a. a reflex secretion of adrenaline,

b. formation of an as yet unknown organic compound probably in the muscles.

The two actions combined produce the fall in plasma inorganic phosphate.

(47) Experiments showing the effect of adrenaline on the plasma inorganic phosphate of Xenopus are required to confirm this hypothesis as to the effect of insulin on Xenopus.

(48) The rise of plasma inorganic phosphate during the hypoglycaemic state is ascribed to the cessation of phosphate withdrawal for metabolic purposes and since the call on inorganic phosphate is no longer present the level rises in the blood. Increased excretion of phosphate is not sufficient to prevent a rise of blood inorganic phosphate.

(49) Changes in blood glucose are directly proportional to changes in plasma inorganic phosphate. On pancreatectomy both rise and with insulin injection both fall.

(50) .....

(50) The secondary fall of plasma inorganic phosphate consequent on injection of anterior pituitary extract may possibly be due to reflex stimulation of the pancreas by the hyperglycaemia with the discharge of insulin into the blood-stream.

(51) Injection of adrenaline into normal animals produce a fall in plasma inorganic phosphate. This is followed by a temporary rise above the pre-injection level.

(52) A direct relationship between adrenaline and phosphorus metabolism is suggested.

(53) The fall in plasma inorganic phosphate is attributed to withdrawal of inorganic phosphate for the formation of hexose phosphate. The accumulation of hexose phosphates in the body is sufficient to account for the fall in blood and urinary inorganic phosphates.

(54) The subsequent rise in plasma inorganic phosphate is attributed to an overswing phenomenon. The initial action of adrenaline is to produce a fall in inorganic phosphate and a rise in calcium follows. The H ion concentration is upset and an acidosis results. This is followed by a slow compensatory phase in which the inorganic phosphate of both blood and urine rise and calcium falls. An over-swing occurs leading to a transient rise of plasma inorganic phosphate above the normal level.

(55) Reflex secretion of adrenaline after insulin injection

may .....

may contribute to the fall in plasma inorganic phosphate occurring after the injection of insulin.

(56) Injection of insulin into hypophysectomised animals produces a decreased diminution of plasma inorganic phosphate as compared with operative controls. It is suggested that the cause is the inability of adrenaline, reflexly secreted, to assist in lowering the plasma inorganic phosphate. This may possibly be due to abnormal stability of muscle glycogen and consequently of the organic phosphorus compounds.

(57) Insulin depresses plasma inorganic phosphate partly by a direct action on phosphorus metabolism. The complete effect in normal animals is a dual one:-

a. reflex secretion of adrenaline,

b. formation of an as yet unknown compound,

The two actions combined produce the whole fall in plasma inorganic phosphate. In hypophysectomised animals the action of adrenaline is cancelled out so that only the formation of organic compound takes place and as a result the demand on inorganic phosphate is less than in normal animals where both mechanisms are at work.

(58) The anterior pituitary is necessary for the complete action of adrenaline on plasma inorganic phosphate. Absence of the anterior pituitary leads to no initial fall of plasma inorganic phosphate after adrenaline injection.

(59) .....

(59) This is attributed to an abnormal stability of muscle glycogen to the mobilising action of adrenaline. Small changes in hexose phosphate occur and consequently little extra demand for inorganic phosphate takes place.

(60) The secondary rise in plasma inorganic phosphate after adrenaline injection is presumably not due to changes in muscle hexose phosphate but may possibly be due to slow compensatory processes adjusting the relationships of the various plasma constituents. The anterior pituitary is not required for this.

(61) Injection of anterior pituitary extract into ovariectomised animals produces a fall in plasma inorganic phosphate similar to that occurring in operative control animals. The secondary fall in plasma inorganic phosphate consequent on anterior pituitary injection is therefore, unlikely to be due to stimulation of the gonads with the liberation of gonadal hormone into the blood-stream.

(62) Injection of progesterone and oestradiol benzoate into hypophysectomised animals produces a fall of plasma inorganic phosphate similar to that occurring in operative controls and in normal animals.

(63) The anterior pituitary is therefore not involved in the action of progesterone or oestradiol benzoate on the plasma inorganic phosphate.

(64) .....

(64) The action of progesterone on plasma inorganic phosphate is independent of an associated action on ovulatory processes as ovulatory processes have nothing to do with the fall in plasma inorganic phosphate.

(65) Injection of parathyroid hormone has the same effect on the plasma inorganic phosphate of hypophysectomised, operative control and normals, namely an initial fall followed by a subsequent rise above the normal level.

(66) It is therefore suggested that the parathyroid hormone acts by directly affecting phosphorus metabolism without the intervention of the anterior pituitary.

(67) Injection of thyroxine has the same effect on plasma inorganic phosphate of hypophysectomised, operative control and normal animals, namely a fall followed by a subsequent return to the normal level.

(68) It is therefore suggested that thyroxine acts by directly affecting phosphorus metabolism without the intervention of the anterior pituitary.

(69) The fall in plasma inorganic phosphate following anterior lobe hypophysectomy cannot be due to hypofunction of the pancreas because:-

a. pancreatic deficiency leads to a rise in plasma inorganic phosphate,

b. injection of insulin into anterior lobe hypophysectomised .....

tomised animals does not restore the plasma inorganic phosphate level but produces a further depression.
It may possibly be due to the unopposed action of insulin due to lack of the anti-insulin factor from the pituitary gland.

(70) The fall in plasma inorganic phosphate following anterior lobe hypophysectomy cannot be due to hypofunction of the adrenal medulla because:-

a. adrenaline produces a fall in plasma inorganic phosphate.

b. injection of adrenaline into anterior lobe hypophysectomised animals does not restore the inorganic phosphate level.

(71) It cannot be due to hypofunction of the gonads because hypofunction of the gonads produces no change in plasma inorganic phosphate.

(72) It cannot be due to parathyroid deficiency either because:-

a. injection of parathyroid hormone produces a fall in plasma inorganic phosphate and an opposite effect might be expected with hypoparathyroidism (see introduction)

b. injection of parathyroid hormone into anterior lobe hypophysectomised animals does not restore the plasma inorganic phosphate but produces a further depression.

(73) It cannot be due to thyroid deficiency because:-

a. .....

a. thyroid deficieney produces no change in plasma inorganic phosphate.

b. injection of thyroxine into anterior lobe hypophysectomised animals does not restore the plasma inorganic phosphate but produces a further depression.

(74) Possible modes of action of the anterior pituitary on the plasma inorganic phosphate are therefore:-

a. by altering the plasma constituents.

b. by affecting carbohydrate and consequently phosphorus metabolism either directly or through the adrenal cortex.

c. by raising the renal threshold to phosphate.

(75) It is considered most probable that the pituitary directly affects the plasma constituents and the presence of the anterior lobe is necessary for the maintenance of a normal plasma inorganic phosphate level.

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INTRODUCTION.

Before recording the results obtained from experiments performed in this investigation, it might be appropriate to review briefly the known factors which affect phosphorus metabolism in animals and to discuss their significance. The level of the blood inorganic phosphate may be used as an index to the metabolic changes in the normal and abnormal states experimentally created. It must be remembered, however, that factors affect the level of the blood inorganic phosphate indirectly i.e. by altering the degree and extent of absorption or excretion of phosphorus and not by mobilisation of this element from depots such as the bones, soft tissues and organic phosphorus compounds. Ultimately an animal is dependant on its supply of phosphorus on what is contained in the ingested food.

It is proposed, therefore, to discuss firstly the level and state of phosphorus in the blood particularly as far as the endocrine glands are involved, for it is with this subject that this work is mainly concerned, and secondly to describe briefly the factors influencing the absorption of phosphorus from the alimentary canal and the excretion by the renal and faecal routes.

1. Factors influencing the level and state of phosphorus in the blood stream.

(a) State of phosphorus in the blood stream

Four phosphorus compounds exist in the blood (Kay and Byrom, 1929), of which only one is inorganic phosphate (ortho-phosphate) the others being in organic combinations as:-

(1) Ester phosphorus e.g. glycerophosphates, phosphoglycerates, hexose phosphates and nucleotides.

(2) Lipide phosphorus e.g. lecithin, cephalin and sphingomyelin.

(3) Nucleic acid phosphorus.

According to most observers the inorganic phosphorus is equally distributed between cells and plasma (Best and Taylor, 1937).

The quantity of organic phosphorus in the blood is many times greater than that of the inorganic. In whole human blood the organic phosphorus amounts to from 35 - 40 mgs./100 ml. (inorganic Phosphorus 3 mgs./100 ml.) and most of it is in the red cells. Laking of the blood or allowing whole blood filtrates to stand leads to fairly rapid conversion of organic to inorganic phosphorus by the phosphatase present in blood.

In the following table is given the distribution of inorganic, ester and lipide phosphorus in normal human blood (Best and Taylor, 1937).

Phosphorus in Whole Blood. (mgs./100 ml.)

(1)	Total Phosphorus	40
(2)	Total acid soluble - 90 per cent in cells	- 27
(3)	Inorganic - in cells and plasma	- 3
(4)	Ester - practically all in cells	- 24
(5)	Lipide - in cells and plasma	- 13

With the exception of the inorganic fraction little is known concerning the variations in blood phosphorus. Inorganic phosphorus is very mobile and the enzyme phosphatase in the blood can rapidly hydrolyse the organic forms and can convert them to the inorganic form.

(b) Species Difference.

The level of plasma inorganic phosphate is fairly constant in the human subject, being of the order of 3 - 5 mgs/100 ml. plasma. This is the normal range for dogs (Marenzi and Gerschman, 1935; Chaikoff et al, 1935). Rabbits have a more unstable mineral metabolism and exhibit a wider range (Sahyun, 1933). In the clawed toad, however, it will be shown that higher figures are obtained varying from 4.4 - 7.2 mgs/100 ml. according to the season. A sex difference is also observed in this species the females having a higher value than the males. This is interesting to note, as females Xenopus laevis and

Gallus .....

Gallus domesticus (Charles, 1931; Shapiro, 1933b) have a significantly higher serum calcium level than the males.

(c) Age

In man the blood inorganic phosphate is higher in infants than in adults (Hawk, 1936) and the serum calcium behaves in the identical manner. Both calcium and inorganic phosphate gradually decrease with age. In healthy non-pregnant women Hull and Bill (1933) found the normal range of serum calcium was 10 - 11.5 mg/100 ml. Inorganic phosphate had the value of 3.2 - 4.4 mg./100 ml. for a woman under the age of 30 and 2.6 - 4.2 mg./100 ml. for women over the age of 30. (This problem was not investigated in Xenopus. Only adult toads were used during the investigation.)

(d) Seasonal variations.

Information under this heading is not very exact. The plasma inorganic phosphate level of children rises during summer and falls during late winter. Rickets has its greatest incidence during late winter. These findings may be correlated with the degree of exposure to solar ultraviolet rays (Hawk, 1936). Bekwin and Bekwin (1929) have found that calcium behaves in a similar manner. Hull and Bill (1934) found that the inorganic

phosphate....

phosphate level of healthy non-pregnant adult women was not affected by the season.

In the clawed toad Shapiro (1933b) has shown that the serum calcium of female toads is lowest in autumn and early winter and highest in late winter and early spring i.e. during the rainy and breeding season. The serum calcium remains at a high level throughout the spring and summer months. In the sequel data will be described with reference to a detailed investigation into the seasonal changes in plasma inorganicphosphate of Xenopus laevis, both male and female extending over a period of 12 months.

(c) Serum Calcium.

It has long been recognised that plasma inorganic phosphate is inversely related to serum calcium. Intravenous injection of sodium phosphate (Binger, 1917) produces hypocalcaemia in dogs as well as tetany. Ganterow (1931) found a rise in calcium and a fall in inorganic phosphate after insulin injection. Parathyroid injection at first produces a fall in inorganic phosphate and a rise in calcium (Albright and Ellsworth, 1929). Peters and Eisenson (1929) in human subjects, Fraser (1932) in sheep, McLean and Minrichs (1935) in the isolated frog heart have emphasized the inverse relation between calcium and inorganic phosphate.

In.....

In the sequel the correlation between the seasonal variation in serum calcium with the seasonal variation in plasma inorganic phosphate is shown and discussed.

(f) Acid base equilibrium.

Phosphorus plays an important role in the maintenance of the acid base equilibrium of the blood and tissues and forms one of the important buffer systems. In the plasma the sodium acid, alkaline phosphates in the corpuscles the potassium acid and alkaline phosphates are in equilibrium with each other. If excess acid say lactic acid enters the blood one of the reactions taking place is the following:-

$HL + Na_2HPO_4 \longrightarrow NaL + NaH_2PO_4$

The ratio NaH_2PO_4/Na_2HPO_4 is readjusted by the excretion of the excess acid phosphate in the urine (Best and Taylor, 1937). The H ion concentration of the urine is determined mainly by the proportion of acid and alkaline phosphates present. In the blood $\frac{NaH_2PO_4}{NaHPO_4} = \frac{1}{4}$ whereas in the urine it is reversed and $= \frac{4}{1}$

In consequence changes in inorganic phosphate concentration may occur secondary to changes in H ion concentration of the blood. The blood always tends to maintain the acid base equilibrium at a constant and this may be partly achieved by changes in norganic phosphate concentration.

(g) Carbohydrate.....

(g) Carbohydrate Metabolism.

Phosphorus is intimately connected with the metabolism of carbohydrate and changes occurring in blood inorganic phosphate are frequently secondary to processes involved in carbohydrate metabolism.

(i) Absorption According to Verzar and McDougall (1936) glucose and galactose are phosphorylated in the intestinal cells being absorbed as soon as they are set free in the intestine. As a result the diffusion gradient is kept high. Similarly reabsorption of glucose from the renal tubules is carried out through the same phosphorylating mechanism. Phlorrhidzin which interferes with phosphorylation inhibits intestinal and renal absorption of glucose.

(ii) Oxidation When glucose is oxidised in the body it is probably first phosphorylated to form hexose phosphates. Hexose phosphates are then probably split into triose phosphate which are subsequently broken down and eventually glucose is oxidised to carbon dioxide and water. Although the intermediary processes of carbohydrate oxidation are not yet definitely established it is highly likely that phosphorus is intimately concerned (Bose and De, 1939).

(iii) Muscle metabolism The breakdown of glucose to lactic acid and the subsequent resyntheals is brought about by the intermediation of phosphorus. At first fructose

di-phosphate ....

di-phosphate is formed and eventually through various phosphate esters lactic acid is produced (Green, 1937). Creatine phosphate appears to serve as a reservoir for phosphorus (Parnas and co-workers, 1936a, 1936b, Needham, 1937).

Since phosphorus is required for so many stages of carbohydrate metabolism it is not difficult to understand that endocrine secretions affecting carbohydrate metabolism involve phosphorus metabolism.

(h) Fat metabolism

Most of the more complex fatty substances contain phosphorus as an integral constituent e. g. lecithins, cephalins and cerebrosides.

(i) Absorption Verzar and McDougall (1936) have shown that absorption of fats from the intestine depends on phosphorylating processes. Anything opposing phosphorylation opposes fat absorption. Absorption of fatty acids depends on a continuous synthesis and breaking down of phospholipides.

(ii) Oxidation Sinclair (1937) has found that phospholipides serve as a vehicle for the transport of fatty acids and are intimately concerned in the intermediary oxidation of fats. Certain Phospholipides contain highly unsaturated acids which form an essential part of the cell composition.

(i) Protein .....

(i) Protein metabolism

Phosphorus appears in a large number of proteins and thus must play an integral part in the structure of protoplasm. It is found in nucleoprotein and nucleotides. The importance of nucleotides in the cellular enzyme complexes, particularly the co-enzyme systems has been emphasized by Dixon (1937, 1939) and Linderstrøm-Lang (1939).

(j) The Parathyroid Glands.

The effect of the parathyroids on the metabolism of calcium and phosphorus has frequently been reviewed (Collip, 1926b; Dragstedt, 1927; Thomson and Collip, 1932, 1934; Cameron, 1936).

Considerable species difference exists in the response to parathyroid injection and removal. To reproduce effects produced by smaller doses on one particular animal requires enormous doses in others and some species are extremely resistant to the action of parathyroid extracts. There is also an enormous variation in the response of different animals to different doses of parathyroid hormone injections. Single small doses may produce no objective effects whereas repeated injections at short intervals of time produce marked effects.

(1) EFFECTS OF EXTIRPATION OF PARATHYROID GLANDS.

Accessory parathyroid tissue is exceedingly common and removal of the parathyroid glands may lead only to a

temporary .....

temporary deficiency in parathyroid secretion. In the earlier experiments the presence of accessory parathyroid tissue was not recognised. The results of earlier experiments, where serial sections were not cut to exclude their presence, cannot be considered reliable.

Parathyroidectomy leads to an abrupt and rapid fall in the concentration of calcium in the serum. (Mac Callum and Voegtlin, 1909). This is not due to increased calcium excretion as was thought at the time, since the urinary calcium output is actually reduced (Cooke, 1910; Greenwald and Gross, 1925a). An increase in blood inorganic phosphate has been reported by Hastings and Murray (1921), Elias and Weiss (1922), Salvesan (1923), Weaver and Read (1929), Esau and Stoland (1930a) in dogs. Gross and Underhill (1922) found no change in the total phosphorus in parathyroidectomized dogs. Inouye (1924) showed that the inorganic phosphate content of serum was always increased in thyroparathyroidectomised dogs even if the animals were kept free from tetany. The hyperphosphataemia gradually returned to normal in the course of time(vide supra). In hypoparathyroid tetany serum calcium is low, inorganic phosphate high, calcium and inorganic phosphate excretion low (Aub et al, 1932). Parathyroidectomy in the

rabbit .....

rabbit produced a very high serum inorganic phosphate value (Collip, 1926c) and if parathyroid hormone was given directly following the operation the rise was prevented. Shortly after the removal of the glands (Collip 1926a) blood calcium started to fall and inorganic phosphate did not change. Later inorganic phosphate rose and in tetanic state was greatly increased.

Striking changes in phosphorus excretion were described by Greenwald and Gross (1925a), Greenwald (1926), a marked diminution occurring. This is not accounted for by an increased organic phosphate excretion in urine nor by an increased excretion in the faeces (Greenwald, 1911). This fall in excretion of phosphorus is characteristic of parathyroidectomy. In a case of idiopathic hypoparathyroidism Albright and Ellsworth (1929) found that the urinary phosphorus excretion was normal but the serum inorganic phosphate was very high. Both the serum calcium and the calcium excretion were low.

(11) EFFECT OF PARATHYROID EXTRACTS.

(a) Single Injection in Dogs.

"The most striking and most conspicuous result of the subcutaneous or intramuscular injection of a potent parathyroid extract in a normal dog, is the rise in serum calcium. The amount of rise is generally proportional to the dose. It occurs gradually and attains its maximum in about 12 - 18 hours". (Thomson and Collip, 1932).

Concomitant ....

Concomitant with the rise in calcium there is a decrease in plasma inorganic phorphorus, though the acidosis may mask the calcium phosphate relationship and at times not only the organic phosphorus but the PO_4''' ions may increase. Fisher and Larson (1925) produced a rise in calcium and inorganic phosphorus of serum in normal and in parathyroidectomised dogs with parathyroid injections.

An increase in the excretion of calcium and phosphorus in the urine and in the volume of urine excreted may be seen in the first hour before any change in the composition of the blood occurs (Greenwald and Gross, 1925b, 1926b; Albright and Ellsworth, 1929), and the increased excretion continues for some time.

(b) Single Injection in Man

The response of the human subject to parathyroid hormone appears to me similar to that of the dog (Herxheimer, 1927). The fall in plasma inorganic phosphate is as rapid though not as great as the rise in calcium (Brehme et al, 1927). Albright and Ellsworth (1929) investigating a case of idiopathic hypoparathyroidism showed that the administration of parathyroid extract produced an immediate rise in urinary phosphate, the serum inorganic phosphate consequently fell and the serum calcium rose.

Cessation of the injection led to a rise in serum inorganic

phosphate ....

phosphate and a fall in serum calcium. The urinary phosphate and calcium fell simultaneously. Ellsworth and Howard (1934) injected parathyroid hormone (40 units Collip) intravenously and noted an immediate outpouring of phosphorus in the urine; the plasma inorganic phosphate showed a slight fall, the serum calcium in several instances showed a fall instead of the expected increase. Gilligan et al (1933) injecting parathyroid hormone intramuscularly into oedematous patients found the calcium content of oedema fluid increased and the decrease in serum inorganic phosphate was reflected by a quantitatively similar decrease in inorganic phosphate content of the oedema fluid. In contrast to this the calcium and inorganic phosphate content of cerebro-spinal fluid did not change significantly in spite of marked changes in blood. Hanson et al (1936) showed that injection of parathyroid hormone into patients suffering from osteogenesis imperfecta produced a fall of serum inorganic phosphate just as in normal subjects. Parathyroid hormone produced a negative balance of phosphorus and calcium.

(c) Single Injection in other Species.

The response of cats and rabbits is very variable and cats appear to be extremely resistant to parathyroid administration (Bauer et al, 1929). The serum calcium of a rabbit is extremely variable and this animal is very unsatisfactory for this type of work. Bodansky et al (1930)

found .....

found that with a single injection the serum inorganic phosphate of young fasted guinea-pigs showed a marked rise. This indicated overdosage. In fed animals little change or a fall below the normal range occurred. In adult animals the effect was less well marked but serum inorganic phosphate rose after injection. Injection of massive doses to normal or parathyroidectomized rabbits, on the other hand, had no effect on the blood inorganic phosphate (Collip, 1926a). Rabbits, thus differ from dogs or cats and for this reason overdosage phenomena are not produced. In the rat Shelling et al (1933) found that parathyroid hormone injections invoked a moderate hypercalcaemia and hyperphosphataemia. The hyperphosphataemia depended upon the dose of the hormone and the phosphorus intake e.g. a stock diet with larger doses of parathyroid hormone increased the serum inorganic phosphate 100 per cent but with a rachitogenic diet the serum inorganic phosphate remained at rachitic levels. Pugsley (1932) found that parathyroid hormone injections into rats led to an increase in serum and faecal calcium and in urinary calcium and phosphate. Parathyroid hormone raised the blood calcium of hypophysectomised or thyroidectomized pigeons just as it did in normal animals (Riddle and Dotti, 1935).

In all these experiments it will be noticed that animals fairly high in the developemental scale have been used

viz ....

viz. pigeons, rats, rabbits, cats, dogs and man.

Very little has been done on the simpler species. It would be interesting to note the effect of parathyroid on animals low down in the developmental scale such as fishes, amphibians and reptiles. In the sequel experiments on the clawed toad will be described.

(d) Repeated injection in Dogs.

With a single injection of parathyroid hormone in normal dogs a gradual rise in blood serum calcium occurred (Collip, Clark and Scott, 1925). Two injections into normal or parathyroidectomized animals produced varying effects depending on the time interval. If the second dose was given early the general character of the curve remained unaltered but if it was given at the peak of the calcium level, the dip in the curve resulted before it took its effect. The cumulative effect may lead to a strikingly marked hypercalcaemia. Successive injections of extracts into normal dogs produced an increase in calcium to a very high level. The premortal fall of serum calcium was accompanied by a sharp rise in inorganic phosphate suggestive of a protective response to control the extreme hypocalcaemia. Serum inorganic phosphate during parathyroid hormone overdosage was high if the blood calcium level was high and if a fatal issue resulted the serum inorganic phosphate rose to an extremely high level.

The blood inorganic phosphate (Collip and Clark, 1925) may show very littlefluctuation during the period of calcium increase, but with the establishment of a marked hypercalcaemia the inorganic phosphate is increased progressively and has soared to very high levels in the last few hours of life.

The following is the sequence of events (Thomson and Collip, 1932). With continued administration of parathyroid extract to dogs the excretion of phosphorus calcium and nitrogen in the urine increases promptly at the beginning of the experiment but declines sharply if the experiment is interrupted. The extra phosphorus excreted is not wholly derived from protein and bone (Greenwood and Gross, 1925b). At about the 24th hour the non-protein nitrogen and the urea of the blood begin to rise and increase steadily throughout the experiments from then onwards, the plasma inorganic phosphate, which at first decreases slightly, later increases in a curve which runs parallel to that of the blood urea. It is therefore likely that the increases are both attributed to failing renal function especially since it appears that the urinary excretion of phosphorus decreases at this time. The fall of calcium is probably due to the restriction of calcium solubility by the rising phosphorus. The theory that the high concentration of calcium in serum leads to renal damage receives further

confirmation ....

confirmation from the work of Albright et al (1929). On administration of parathyroid hormone to normal human subjects on a low calcium intake, the negative balance became still more negative, the excretion of phosphate in the urine (not faeces) increased and a fall in serum inorganic phosphate resulted. When the serum calcium rose above 14 mgs / 100 ml., the excretion of phosphorus failed to keep pace with this and was diminished, the concentration of inorganic phosphorus in the serum then increasing. Cantarow et al (1935) gavevery large doses of parathyroid extract to dogs in repeated injections totalling about 30,000 units. A marked rise in serum inorganic phosphate occurred in every case. This was preceeded in some by a fall in serum inorganic phosphate. Shelling et al (1938) showed that the toxic effect of parathyroid overdosage was due to dehydration resulting from the marked diuresis. Saline and glucose injected with parathyroid hormone kept their animals alive.
Chown et al (1936, 1937) in rats, and Cantarow et al (1938) in dogs have produced various lesions in the kidney with large doses of parathyroid hormone but these lesions are not specific.

(c) Repeated injection in other species.

In cats, rabbits, rats and guinea pigs, the toxic effects of parathyroid hormone injection are much more

difficult ....

difficult to elicit (Thomson and Collip, 1932). Bodansky et al (1930) found that daily doses of 10 units / day for 2 weeks produced no change in the inorganic phosphate nor in calcium of guinea pigs but if 40 units /day was injected hypercalcaemia and hypophosphataemia resulted.

In the human cases of hyperparathyroidism reported in the literature 50 per cent showed a constant hypercalcaemia and 50 per cent a constant hyperphosphataemia. An increased phosphatase activity was present in every case of classical hyperparathyroidism (Gutman et al., 1936).

All the effects of parathyroid overdosage can be imitated by injecting acid sodium phosphate (NaH_2PO_4) and calcium chloride. (Collip, 1926b). Parathyroid hyperplasia can be produced by parenteral administration of phosphate (Drake et al, 1937).

111) MODE OF ACTION OF THE PARATHYROID GLANDS

The suggestion that the mobilisation of calcium is due merely to an acidosis cannot be accepted. A slight rise in potassium and magnesium has been reported (Scholtz, 1931), but this effect is transient and has vanished before the rise in calcium approaches its maximum (Collip and Clark, 1925). Mc Lean et al (1930) attribute the function of the parathyroids to the maintenance of the calcium ion concentration of the plasma at the physiological level, removal leading to the reduction of ionized calcium, injection of parathyroid hormone leading to an

increase of ionized calcium in the serum. It has been thought by some (Cameron and Moorhouse, 1925) that the hormone controlled the formation of a specific non-diffusible organic calcium compound. Increase of the organic compound in the blood caused a corresponding increase in ionic calcium which was furnished by the bones. After parathyroidectomy conditions were reversed, a fall in concentration of the compound and a reduction in ionic calcium resulted. Another view (Greenwald, 1924, 1926) was that the hormone itself increased the solvent power of the plasma for calcium.

Albright, Bauer, Ropes and Aub, (1929) injected parathyroid hormone into normal human subjects and showed that the inorganic phosphate level of the blood was primarily lowered by parathormone. If the serum calcium rose above a critical level, the urinary phosphate excretion fell, and blood inorganic phosphate rose, the primary effect of parathormone being to increase the urinary phosphate excretion and this precedes the increase in calcium excretion. As the serum inorganic phosphate falls after parathyroid administration, the serum calcium rises and urinary phosphate is increased. Greenwald and Gross (1925a) have pointed out that after parathyroidectomy the decline in phosphate excretion was more striking than the decline in calcium excretion and that in parathormone overdosage

the .....

the extra excretion of phosphorus was greater than that to be expected from calcium and nitrogen excretion. Greenwald and Gross (1926) found that the extra phosphate excreted was not wholly derived from $Ca_3(PO_4)_2$ nor from protein.

Albright and Ellsworth (1929) put forward the following hypothesis. When parathyroid hormone is administered the equilibrium of the body fluids is upset in such a way that an increased phosphate excretion is a necessary result. The cause of increased phosphate excretion is not known, but as a result of the increased phosphate excretion, the body fluids become depleted of inorganic phosphate. The falling inorganic phosphate is evidence of this. As the inorganic phosphate and consequently phosphate ions in serum fall, there is a tendency to unsaturation of the calcium phosphate of the blood. The tendency is met by a mobilisation of calcium phosphate from the bones. Thus a deficit of phosphate ions is supplied by calcium and phosphate ions. Consequently serum calcium rises. With a rise of serum calcium, provided the level is not below the threshold for calcium excretion there is a rise in urinary calcium output. Thomson and Collip (1932) were unable to subscribe to this view and showed that the serum calcium and inorganic phosphate do not behave as if in equilibrium with calcium phosphate. For example the

inorganic .....

inorganic phosphate drops after insulin injection and yet there is little compensatory increase in calcium.

Albright, Bauer, Cockrill & Ellsworth (1931) state that as the serum calcium varies between the level in the hypoparathyroid state and the normal state and vice versa, the phosphate varies inversely so that the product of Ca X P remains constant, except at very high levels of serum calcium resulting from hyperparathyroidism. Then the serum inorganic phosphate no longer falls, but abruptly rises suggesting a secondary phenomenon. Low calcium levels in hypoparathyroidism are associated with elevated serum phosphate levels, high serum calcium levels in hyperparathyroidism is associated only with a moderate reduction of serum inorganic phosphate values.

Albright et al (1932) showed that injection of large amounts of phosphate in clinical hyperparathyroidism results in almost complete absorption of the phosphate in the blood stream. Absorbed phosphate is rapidly excreted by the kidney suggesting that the depressed serum inorganic phosphate level in hyperparathyroidism is not below the threshold for phosphate excretion but that the threshold itself is low. In spite of the effort of the kidneys to excrete phosphate the serum inorganic phosphate can be made to rise and serum calcium to fall. The fall in calcium does not correspond to the rise in inorganic phosphate and the product Ca X P increases its value.

The .....

The net result is a positive balance of calcium and inorganic phosphate values in the direction of normal.

Brull (1936) perfused the kidneys of dogs injected with parathormone and found that the kidneys took up parathyroid hormone from the blood and parathormone acted by lowering the renal threshold for phosphate. Brull (1937) found that hypophysectomy in dogs, led to a rise in blood inorganic phosphate in the first hour as a result of a rise in the renal threshold for phosphate. Injection of gonadotrophic, thyrotrophic Pregnancy Urine and posterior lobe extracts failed to restore the secretion. If parathyroid was injected the excretion of phosphates was increased as the threshold was lowered by parathyroid hormone injection.

Collip et al (1934) on the other hand, claimed that parathormone exerts its effects on calcium metabolism by a direct action on the bones. Kidneys of rats were removed and the animals injected with parathyroid hormone. The femurs showed marked signs of osteoclastic bone resorption. The action of parathyroid hormone on bones was independant of any direct influence it may have on the renal threshold for phosphate. Pugeley and Selye (1933) showed that continuous administration of parathormone produced the formation of osteoclasts and hypercalcaemia resulting in increased calcium excretion. This phase is followed by the appearance of numerous osteoclasts and decreased calcium excretion. They concluded that calcium was mobilized by

cellular .....

cellular activity and not by changes in the Ca/P relationship of blood. Schour and Ham (1934) nevertheless consider the appearance of osteoclasts to be the result of absorption of calcium from bone rather than these elements are active agents. Ellsworth and Futcher (1935) found that administration of parathyroid extracts results in a marked rise in serum calcium in nephrectomized dogs. They thus concluded that the elimination of phosphorus was not necessary for the production of an elevated serum calcium. Tweedy et al (1937), on the contrary, showed that injection of parathyroid hormone into bilaterally nephrectomised dogs led to little change in calcium and a rise in inorganic phosphate. In unilaterally nephrectomised dogs the usual rise in calcium and fall then a rise in serum inorganic phosphate occurred. Tweedy and McNamara (1937) found no change in serum calcium in nephrectomized rats injected with parathormone. McJunkin, Tweedy and McNamara (1937) confirmed this finding and showed that although the rise in serum calcium did not occur a pronounced osteoclastic resorption of bone took place. They concluded that the amount of extraskeletal calcium liberated by the action of parathyroid hormone did not accumulate in the blood because much of it was eliminated by the intestinal route. Pugsley (1932) using small doses of parathyroid extract found increase of calcium excretion in urine and faeces.

Goadby and Stacey (1934, 1936), Goadby (1937) support the hypothesis that parathyroid hormone acts directly on the kidney to produce an increase of phosphate excretion. When parathyroid hormone is injected into chronic nephritics it fails to cause the same increased excretion of phosphorus in the urine as occurs in normal persons. In patients suffering from acute nephritis a smaller phosphate diuresis occurred after parathyroid hormone injection than when they had recovered from the disease. Gilligan et al (1933) showed that the maximum fall of serum inorganic phosphate in oedematous subjects occurred 24 - 48 hours before the maximum increase in calcium concentration and cite this as being in accord with the concept that the primary action of parathyroid hormone is the decrease of plasma inorganic phosphate.

(k) Vitamin D

Higgins and Sheard (1928), Hess (1929) and Morgan and Garrison (1930) have shown that vitamin D and parathyroid hormone affect the level of serum calcium and inorganic phosphate but in several respects their mode of action differs.

Parathyroid hormone administration to rats (Pugsley, 1932) resulted in a marked increase in urinary calcium and a slight increase in urinary phosphate, a marked increase in faecal calcium but no change in faecal phosphate.

Irradiated .....

Irradiated ergosterol led to a definite decrease in faecal calcium and phosphate and an increased urinary calcium. Again the parathyroid hormone, while causing an increase in serum calcium level leads to a primary decrease in serum inorganic phosphate whereas both serum calcium and serum inorganic phosphate are increased after administration of vitamin D.

Jones and Robson (1933) gave overdoses of irradiated ergosterol to rats for two months and found very marked degenerative changes in the femora of their animals, the bones being soft and fragile. The destruction was apparently due to osteoclastic activity. Not only inorganic constituents but organic material was removed from the bones.

Vitamin D is probably concerned in a triple action on the metabolism of calcium and phosphorus. It takes part in the absorption and excretion of calcium and phosphorus (as will be discussed in the sequel) as well as in the intermediary metabolism of these elements (Clouse, 1932). Hansman (1934), however, considers that vitamin D does not act by increasing the absorption of phosphate nor by changing the calcium and phosphorus levels of the serum. He suggests that its action is to make calcium available for tissue metabolism.

1. <u>The Thyroid</u> .....

(1) The Thyroid gland.

The thyroid gland may influence phosphorus metabolism either through its general effect on metabolism and carbohydrate metabolism in particular, or through its effect on the mineral metabolism of bones.

(1) General metabolism

It is generally considered that the normal function of the thyroid gland is linked with the oxidative processes of the body cells (Cameron, 1936). Thyroid stimulates metabolism in general but since phosphate and carbohydrate metabolism are so intimately connected the effect on carbohydrate metabolism is here of more concern. Thyroxine has no immediate effect on blood sugar but large doses when administered daily for some time, produce profound changes in carbohydrate metabolism. At first a slight hyperglycaemia and glycosuria may appear adrenaline producing a greater hyperglycaemic response and insulin a less profound hypoglycaemia than normally (Burn and Marks, 1925). When more thyroxine is given the liver glycogen (Cramer and Krause, 1912) and muscle glycogen (Burn and Marks, 1925) become diminished and the animals become very susceptible to insulin and resistant to adrenaline. When the animals die in hypoglycaemia the glycogen content of liver, muscle and heart is very low. Since different doses of thyroxine produce different effects on carbohydrate metabolism (i.e. moderate doses produce hyperglycaemia,

larger .....

larger doses produce hypoglycaemia) different effects on phosphorus metabolism can be expected. This will be discussed in the sequel.

(11) Mineral metabolism

Changes in the calcium content of the serum in thyroid diseases, thyroidectomy or thyroid administration have been extensively investigated. Leicher (1922) found a diminution of serum calcium after administration of thyroidine (Merck) tablets to patients but "the differences found fall within the normal variations and the number of cases are too few to permit any definite conclusion" (Zwarenstein, 1934), Jansen (1924) and Wade (1929) reported low values of serum calcium in hyperthyroidism. Montanari (1928) found a definite rise.

After thyroid administration to experimental animals an increase in serum calcium has been observed by several investigators (Oltramare, 1924; Rothschild and Jacobsohn, 1927; Nishimura, 1928; Trifon, 1929; Blinoff, 1930; and Horsters, 1930).

Aub et al (1929) in man and Kunde et al (1931) in dogs found no change. The bulk of evidence (Zwarenstein, 1934) indicates that serum calcium is subject to slight variation in thyroid disease and after thyroid administration. That there is a tendency to slightly increased values in hyperthyroidism seems to be fairly well established.

Since the thyroid affects the serum calcium level and

calcium and phosphorus are inversely related, an effect on plasma inorganic phosphate is to be expected. Changes in blood inorganic phosphate, however, following thyroid administration have received little attention. Rothschild and Jacobsohn (1927) and Masters (1930) found a slight fall. Aub, Bauer, Heath and Hopes (1929) administered thyroid and thyroxine to normal patients and found that the serum calcium and inorganic phosphate values remained within the accepted normal limits though a few cases suggested a slight fall in these values during the period of observation. Haddock et al (1937) found normal values of inorganic phosphate and calcium in hyperthyroid patients.

The evidence in regard to the effect of thyroidectomy on serum calcium is contradictory. Normal values have been reported in hypothyroidism (Herzfeld and Neuberger, 1924; Gastex and Schteingart, 1925; Waldorp and Trelles, 1926; Percival and Stewart 1926; McCullagh, 1928; Aub et al, 1929; Ask-Upmark, 1932). Thyroidectomy had no effect on the serum calcium content of cattle (Mug, 1921; Cheymol and Quinquaud, 1932b) nor in dogs and cats (Perelman, 1925). An increase has been reported by Leites (1924), Maxim and Vasiliu, (1926) and Werner (1929) and a decrease by Parhon (1923), Robinovitz (1924), Parhon and Derevici (1926), McCullagh (1928), and Parhon, Derevici and Derevici (1930).

It is difficult to draw any definite conclusion from this mass of contradictory evidence. The majority of

observers .....

observers report a slight decrease or no effect after thyroidectomy.

No change in blood inorganic phosphate nor in calcium was found in thyroidectomised dogs (Gerschman and Marenzi, 1935a).

Changes in excretion of calcium and phosphorus, however, have been well established. Scholtz (1905) found that untreated cretins retained phosphorus and excreted large quantities of the alkaline earths, treatment with thyroid had no effect on the phosphate but markedly reduced the calcium output. Parhon (1912) showed that thyroid administration in rabbits caused a negative calcium balance and the loss of calcium is roughly proportional to the dose of thyroid. Kummer (1917) reported exceptionally large calcium and phosphate losses in a case of exophthalmic goitre. Aub et al (1929) made careful studies of the calcium, nitrogen and phosphate excretion in patients suffering from various thyroid diseases. Calcium and phosphate excretion was markedly increased, the increase in phosphate excretion being quantitatively such as to suggest that most of the calcium excreted came from the tertiary calcium phosphate in the bones. Percival and Stewart (1926) suggested that increased calcium excretion in hyperthyroidism was due to an indirect effect of an increase in metabolic rate. The most likely explanation of the increased excretion of calcium and phosphate in hyperthyroidism is as suggested by Aub and his

co-workers .....

co-workers namely a direct stimulating effect on the calcium phosphate deposits of bones. That the calcium and phosphate of bone is mobile and readily available was demonstrated by Bauer, Aub and Albright (1929). Parhon, Derevici and Derevici (1930) treated dogs with powdered thyroid and thyroxine and in both cases found a definite decrease in the calcium content of bone.

Consideration of the above reveals that a definite change in calcium and phosphate excretion occurs with thyroid administration. Thyroidectomy leads to little change in calcium and inorganic phosphate of blood while thyroid administration leads to a slight rise in calcium and no change or a fall in inorganic phosphate. All the work has been done on man, dogs and other mammals and nothing on animals low down in the developmental scale. Such animals might react more simply to the effects of ablation and injection of extracts of endocrine organs and it would be of interest to investigate their reactions. In the sequel the effect of thyroidectomy and thyroxine injection in the South African clawed toad is discussed.

(m) <u>The gonads</u>.

In eunuchoids and human castrates, as well as in animal experiments, a more prolonged growth of bone occurs suggesting that the gonads are implicated in calcium and phosphorus metabolism. It is probable, however, that the effects of castration .....

castration on the skeleton is indirect being an effect of hypertrophy of the anterior lobe of the pituitary which occurs as a result of castration (Addison, 1917; Engle, 1929; Smith, Severinghaus and Leonard, 1933; Severinghaus, 1937).

(1) Gonadectomy

Conflicting results have been reported in the literature particularly as rabbits have frequently been used as experimental animals. Elinoff (1930), Hogben and Charles (1932) and Cheymol and Quinquaud (1932a) found no change in serum calcium after gonadectomy in guinea-pigs, rabbits and dogs respectively. These authors emphasized that the normal serum calcium values of these animals fluctuated within a wide range. Perelman (1925) found no change in serum calcium of gonadectomised dogs and cats. On the other hand decreases after gonadectomy have been reported by Dalsace andGuillaumin (1925) and Davanso (1929) in women, by Suzuki (1931) and Okazaki (1932) in rabbits and by Harlow and Koch (1937) in rats while increases in blood calcium have been reported by Leites (1924) in rabbits and by Blanchetiere (1925) in women. Leites reported results on only one male and one female. This militates against the acceptance of his findings.

Studies on blood inorganic phosphate are few. Dalsace and Guillaumin (1925) described a fall in ovariectomised human females .....

females and Harlow and Koch (1927) confirmed this in gonadectomised rats.

(11) Injection of extracts

Hirvich and Bodman (1927, 1929) drew attention to several observations indicating that the ovary played an important role in calcium metabolism and emphasized the change in skeleton following gonadectomy, the loss of calcium during menstruation, the high blood calcium after the menopause and the alleged cure of osteomalacia as a result of ovariectomy. They postulated an ovarian parathyroid antagonism. Ovariectomy in rabbits indicated no regular changes in serum calcium the values ranging from 13.9 - 18.0 mgs. Ca/100 ml. serum. A crude alcoholic extract equivalent to 25 gm. ovarian tissue lowered blood calcium 30 per cent in 24 hours, although their normal values varied by more than this. Larger doses by mouth gave similar results. No effects were observed from similar extracts prepared from muscle, pancreas, spleen, kidney and brain. The same results occurred in normal, pregnant, non-pregnant, mature, immature, ovariectomized and male rabbits. More purified extracts injected into men and women produced in most cases a fall in serum calcium.

Harlow and Koch (1937) used more purified and biologically standardized sex hormone preparations on fowl, rats and rabbits. Experiments on fowl produced no consistant changes.

The .....

The pre-injection value was extraordinary variable. In most experiments ovarian extract produced a rise in calcium but this was inconsistent. The average values indicated a rise in blood calcium and a fall in inorganic phosphate but individual birds did not react uniformly. Moreover, olive oil produced similar irregular changes. Rats were no better, similar results being obtained. In rabbits injected with a crude preparation similar to that of Hirvish and Boeman's and also equivalent to 25 gm. fresh ovarian tissue, a rise in blood calcium was obtained in all cases and olive oil was about as efficient as the hormone preparation. The inorganic phosphate tended to be lowered in all cases.

Frei and Emmerson (1930) produced a slight fall in serum calcium in cows injected with a commercial oestrogenic preparation. Dixon (1933) found no appreciable change in serum calcium of dogs injected with ovarian and corpus luteum extracts.

Huey and Marlow (1937) prepared four extracts of pig ovary varying according to the degree of purification and injected these extracts into rabbits. They confirmed the inconstancy and fluctuation of normal serum calcium and inorganic phosphate levels and showed that none of the fractions eliminated during the preparation of the purified extract, nor the purified extract, nor a combination of all these, caused a definite and consistant change in serum calcium and inorganic phosphate.

(iii) Calcium

(111) Calcium and phosphorus during menstruation and pregnancy

Consistent variations in serum calcium and inorganic phosphate have not been found at menstruation. Boynton and Greischeimer (1931) reported a fall in blood calcium during the menstrual or postmenstrual periods in normal women. No cyclic variations were observed in dysmenorrhoeic patients. Hall (1908) found a depressed serum calcium level just before menstruation while Sharlit, Corscaden and Lyle (1927) and Watters (1929) reported a pre-menstrual rise and a menstrual fall of serum calcium. Irregular changes (Hoyn and Haase, 1925; Halamud, 1924) and a steady level during the menstrual cycle (Watchorn, 1926, Beck, 1928; Close and Osman, 1928; Allen and Goldthorpe, 1929; Spiegler, 1931) have been reported.

Excretion of calcium and phosphate during menstruation was found to be unchanged (Sherman, Gillett and Pope, 1916; Rogert and McKittrick, 1923).

Close and Osman (1928) found no change in the blood inorganic phosphate during the menstrual cycle. Hall and Hill (1933) found the normal range of blood inorganic phosphate to be 3.2 - 4.4 mg. / 100ml. for women under the age of 30 and 2.6 - 4.2 mg. / 100ml. for women over the age of 30. No changes during the menstrual cycle were observed.

A diminution of serum calcium in the latter months of pregnancy has frequently been observed in women(Widdows, 1923, 1924; .....

1924; Bogert and Plass, 1923; Bokelmann and Beck, 1928; Bauer, Albright and Aub, 1929; Hellmuth and Timpe, 1930; Adler, 1931; Spiegler, 1931; Merritt and Bauer, 1931; Watchorn and McCance, 1932; Hull and Bill, 1934; Ramsay, Thierens and Magee, 1938), and in cows (Godden and Allcraft, 1932). Hull and Bill (1934) found a distinct seasonal difference in serum calcium but there was also a significant decline in calcium due to pregnancy itself up to 6 - 7 weeks before delivery than a slight rise until delivery followed by a sharp increase which lasted throughout the post-partum period under observation. Inorganic phosphate showed no seasonal variation but owing to the pregnancy itself there was a significant fall in the average values until 11 - 13 weeks before delivery followed by an equal rise until term. Immediately following delivery there was a sharp rise 19 per cent higher than those at the beginning of the study. These high values were maintained during the post partum period under observation. Lehwirth (1936) and Ramsay, Thierens and Magee (1938) also found a fall in inorganic phosphate during the middle months of pregnancy. Meranze, Meranze and Rothman (1937) found high blood phosphatase values during pregnancy and low inorganic phosphate values. Towards the latter months of pregnancy the inorganic phosphate returned to normal levels.

Godden and Allcraft (1932) found an unfailing low inorganic phosphate value before and an immediate and marked rise

after .....

after parturition in cows.

During gestation the foetus withdraws calcium and phosphorus and this probably accounts for the low maternal serum calcium and inorganic phosphate values during pregnancy. The source of the calcium and phosphorus is not the teeth (Hull, Bill and Kinney, 1934), but is apparently the bones (Schmidt and Greenberg, 1935; Hull, 1936.)

(iv) Serum calcium and blood inorganic phosphate in relation to the ovarian cycle in aves and amphibia. Riddle and Reinhart (1926) found that in female pigeons the blood calcium is enormously increased to more than twice the normal value at a time eggs are ready to leave the ovary. The beginning of the increase of calcium concentration in the blood dates 123 hours before the egg-shell begins to form. The high serum calcium is thus not correlated with the large calcium demand for shell formation and the hypothesis suggested is that the very high calcium values accompanying each ovulation period is an expression of increased parathyroid activity. The rise and fall of calcium level of the fowl was found to be associated with distinct histological changes in the parathyroids (Macowan, 1932).

Zwarenstein and Shapiro (1933) working on Xenopus laevis have shown that the serum calcium of female toads is lowest in autumn and early winter and highest in late winter and early spring i.e. during the rainy and breeding seasons, when

ovulation .....

ovulation and oviposition occur. Inorganic phosphate should be inversely proportional to the serum calcium and this will be discussed in the sequel. Up to the beginning of the breeding season there is a striking correlation between the state of the ovaries and the serum calcium. A decline and loss of weight of the ovaries occurs as ova are extruded in large numbers during the breeding season but serum calcium is maintained at a high level. During the summer the ovaries further retrogress and the serum calcium drops. The changes in serum calcium level of Xenopus are therefore very similar to those in pigeons during the breeding season. The serum calcium begins to rise and is already at a high level before ovulation occurs just as in pigeons serum calcium is doubled before the ova have left the ovary.

(n) The Pancreas.

(1) Pancreatectomy The results obtained on the effects of extirpation of the pancreas on blood inorganic phosphate are conflicting. Kerr (1926) found no significant change in guinea pigs whereas Gerschman and Marenzi (1935a, 1935b) obtained a rise.

Von Moraczewski (1897), Mandel and Lusk (1904) and van Noorden (1907) noticed an excessive elimination of phosphorus during clinical observations on diabetics. Falta and Whitney (1908) showed that the phosphate excreted in the urine of depancreatised dogs was three times the normal. The relation

of .....

of high phosphate excretion to diabetes has not been recognised because of the accompanying acidosis. Soskey and Allan (1924) found that the phosphate excretion of diabetic dogs without insulin was very high, insulin caused a decrease in the excretion then an increase of the excretion to the level of the normal animal.

(11) Injection of insulin into pancreatectomised animals

Kerr (1926) produced a marked fall in blood inorganic phosphate in guineapigs although pancreatectomy produced no change.

(111) Injection of insulin. Harrop and Benedict (1923) showed that injection of insulin caused a decrease in the inorganic phosphate content of blood in human diabetics, fasting normal subjects, a fasting dog and fasting rabbits. Almost simultaneously Wigglesworth, Woodrow, Smith and Winter (1923) independently obtained the same results in rabbits. Several investigators have subsequently confirmed this finding. (Briggs et al, 1923, in dogs; Kay and Robison, 1924, in rabbits; Tolstoi et al, 1927, in diabetic patients; Kerr, 1926 in dogs; Ellsworth, 1930 in man; Chaikoff et al, 1935a, in dogs; Bose and De, 1939, in normal and in diabetic subjects;) Barrenscheen and Berger (1926) found no change or a rise on injection of insulin in human diabetics. Blatherwick, Bell and Hill (1924) pointed out that injection of insulin in

human .....

human subjects produced a clear decrease in blood inorganic phosphate the change occurring at the time of hypoglycaemia when carbohydrate metabolism was at its peak. Winter and Smith (1924) in rabbits, and Cadic et al (1925), in dogs, showed that the fall in blood inorganic phosphate was parallel to the drop in glucose but that this was followed by a rapid return of inorganic phosphate to the normal level. Sugar administration in the hypoglycaemic state lowered the inorganic phosphate which had returned to the normal level. Holliger and Hartman (1925) confirmed these findings and showed that insulin produced a fall in blood inorganic phosphate but the inorganic phosphate rose throughout the duration of the hypoglycaemic symptoms i.e. there was no demand for inorganic phosphate during hypoglycaemia. Similarly following starvation of 24 hours, when carbohydrate oxidation was at a minimum the concentration of inorganic phosphate was always increased often more than doubled (Cahyun, 1933, in rabbits.)

Ellsworth and Weinstein (1933) drew attention to the fact that the fall of plasma inorganic phosphate in normal animals was followed by a rise above the normal level. Strand, Anderson and Allcroft (1934) in sheep, and Angelini and Rossi (1937) in children have confirmed this finding. Nasseese and Morera (1934) showed that this rise above normal occurred even though the blood sugar level was still low. Schmidt and

Ssantschian....

Isaacsohian (1927) found the blood inorganic phosphate at first depressed by insulin, but when the animals were in convulsions a marked increase occurred.

Insulininjected with sugar also produced a diminution of blood inorganic phosphate (Perlzweig, Lathan and Keefer, 1923 Barrenscheen and Berger, 1926).

(IV) Ingestion of sugar. Ingestion of sugar lowered the serum inorganic phosphate (Salvesen, 1923). Harrop and Benedict (1924) confirmed this finding and attributed it to a reflex secretion of insulin. Katayama and Killian (1927) showed that in human diabetics the blood inorganic phosphate was decreased in some and increased in others. Bolliger and Hartman (1925) found that in normal dogs sugar ingestion produced a fall in blood inorganic phosphate but this did not occur in diabetic dogs nor in diabetic humans. The deepest point in the inorganic phosphate curve, however, lagged well behind the highest point of the sugar curve. They showed that the diabetic state was demonstrated early in the curve of blood phosphate. Blood phosphates were depressed during carbohydrate metabolism only when insulin was available. The complete absence of pancreatic hormone was shown in the blood phosphate level, which remained unaffected in a straight line during carbohydrate metabolism. Carbohydrates and phosphates were best utilized when there was an excess of each in the circulation. McCullagh (1931) made

inorganic .....

inorganic phosphate curves in human subjects after glucose meals and found that the greatest depression occurred 2 to 3 hours after a glucose meal, always after the high point in the blood sugar curve. Changes in the curve occurred in the diabetic state and many cases of hyperthyroidism showed similar changes.

Pollack (1933), Pollack et al (1934) found a drop of serum inorganic phosphate after intravenous injection of glucose. In completely adrenalectomised animals the drop in serum inorganic phosphate paralleled the normal. In hepatectomised animals the drop occurred sooner and the return to normal was more rapid. In depancreatised animals without insulin there was no drop but the addition of insulin to the injected fluid caused a drop to take place. In demuscled animals there was no drop in phosphate concentration but on perfusing the heart-lung-hind-limb preparation there was a marked drop accentuated by insulin. The total acid soluble phosphorus was increased in the muscle, but not the inorganic phosphate. These experiments indicated that insulin was responsible for the fall in inorganic phosphate after glucose administration and this fall occurred owing to the formation of organic phosphate compounds in muscle. Liver activity was not essential for this fall. Bolliger and Hartman (1925) also observed the fall in animals "whose livers had become reduced to a functionless mass of fat".

Pijoan and Quigley (1937) found that removal of the adrenals did not influence the fall of inorganic phosphate following ingestion of glucose but that insulin had no effect on the blood inorganic phosphate of adrenalectomised animals. They therefore suggested that the two processes, ingestion of sugar and injection of insulin, though both producing a fall in blood inorganic phosphate acted through different mechanisms.

Ellsworth and Weinstein (1933), however, also working with dogs found that insulin produced a more marked depression of serum inorganic phosphate in adrenalectomised than in normal animals. Markowitz (1926) injected glucose into normal animals and obtained a fall in urinary phosphate. This he ascribed to insulin secretion since in pancreatectomised animals urinary phosphate was unchanged when glucose was injected.

Bose and De (1939) after a careful series of experiments on normal and diabetic human patients came to the conclusion that ingestion of sugar lowered blood inorganic phosphate by stimulating the pancreas and producing a reflex secretion of insulin. The secretion of insulin assisted in the formation of a hexose phosphate compound which was an intermediary product of glucose oxidation. In healthy subjects the pancreas was in an efficient condition secreting the extra amount of insulin and thus producing a

fall in blood inorganic phosphate. In diabetic subjects, however, the pancreatic secretion was either deficient or almost absent depending on the severity of the disease. In mild or moderate diabetes the supply of insulin was slow and insufficient and thus a slower and decreased demand for inorganic phosphate occurred. In severe cases of diabetes very little insulin was produced and the change in plasma inorganic phosphate was almost absent. If insulin was artificially supplied to these diabetic subjects a marked change in blood inorganic phosphate occurred.

(V) Excretion of phosphorus with insulin and glucose. Fiske (1921) first drew attention to the fact that there was a decreased output of urinary phosphates following sugar ingestion. This decrease was followed by an increase. Soltney and Allen (1924) pointed out that the depression in urinary phosphates following sugar ingestion was almost as great as that following insulin injection. Insulin injection produced a reduced excretion of phosphates in the urine, but this was followed by an increased excretion, the total output of phosphates being actually increased. This closely follows the fluctuation of blood inorganic phosphate after insulin injection. Insulin injected with glucose produced the same effects as either separately. Blatherwick, Bell and Hill (1924), Harrop and Benedict (1924), Mazzocco and Morera (1924) and Barrenscheen and Berger (1926) have confirmed these results. As a further

example .....

example of the close relationship between inorganic phosphate and glucose, injections of inorganic phosphate produce a fall of blood sugar in human subjects when the blood sugar is above the normal value (Ellis and Weiss, 1922).

(VI) Serum calcium. In view of the inverse relation between calcium and inorganic phosphate it would be interesting to note the effect of insulin on serum calcium. An increase in serum calcium has frequently been reported (Davies, Dickens and Dodds, 1926; Schmidt and Shaatschian, 1927; Brougher, 1927; Ellsworth, 1930; Farquharson and Tibbetts, 1931). Reed (1929) found that insulin relieved the symptoms of tetany in parathyroidectomised dogs.
It is probable that the rise in serum calcium is a secondary phenomenon the primary action being on glucose and inorganic phosphate but owing to changes in acid-base equilibrium the calcium is adjusted to maintain the normal constant.

No experiments on amphibians have yet been performed. It would be interesting to note the effect of pancreatectomy and insulin injection on the blood inorganic phosphate of these simpler animals and in the sequel experiments on the South African clawed toad are recorded and discussed.

(c) The Adrenal glands.

(1) Adrenalectomy. Removal of both adrenal glands produced a marked rise in blood inorganic phosphate (Swingle in cats, 1926; .....

1926; Ellsworth and Weinstein, 1933, in dogs; Strand et al, 1934, in sheep). According to Swingle (1926) the rise in inorganic phosphate following adrenalectomy was one of the "most striking and consistent changes in the blood constituents". In most cases the increase was considerable especially in the pre-comatose stage. The accumulation of inorganic phosphate occurred simultaneously with the development of serious symptoms. It was possible to use the blood inorganic phosphate level as a prognostic sign. A high level invariable indicated that coma and death were but a few hours distant. The increase in inorganic phosphate was not solely a terminal event nor could it be accounted for by the increased blood concentration. The responsible factor was probably not deficiency of adrenaline but secretory failure of the kidney.

Lucas (1926) found an increase in plasma inorganic phosphate in adrenalectomised dogs but as the content of plasma inorganic phosphate varied with food or with laking of the corpuscles the increase was of little significance. Baumann and Kurland (1927) found little change in blood inorganic phosphate content of most of their adrenalectomised cats.

(11) Injection of adrenaline. Perlzweig, Latham and Keefer (1923) showed that subcutaneous injection of adrenaline in normal human subjects produced a fall in blood inorganic phosphate. This effect of adrenaline has subsequently been confirmed by several investigators (Vollmer,

1923 .....

1923, in children and adults; Winter and Smith, 1924, in rabbits; Tolstoi et al, 1924, in diabetics; Woringer, 1924, in dogs; Barrenscheen et al, 1927, in rabbits; Labbe et al, 1931, in man.)

The fall in inorganic phosphate is followed by a prolonged increase (Vollmer, 1923a,b,c) Woringer (1924) confirmed this and showed that if adrenaline was injected during the hyperphosphataemia a second fall in inorganic phosphate could be obtained.

Labbe et al (1931) injected adrenaline intra-muscularly in man and showed that there was no change in total blood phosphate nor in the phospholipide content. The organic phosphates constantly rose while the inorganic phosphate was constantly decreased. He thus concluded that the fall in inorganic phosphate was due to an increased production of organic phosphate.

Puncture of the medulla (Dolliger and Hartman, 1925) also produced a fall in blood inorganic phosphate presumable by stimulating sympathetic nerve tracts and releasing adrenaline into the circulation.

Cori and Cori (1931) showed that subcutaneous injection of adrenaline in rats produced a rise in muscle hexose phosphate, after 15 minutes. This reached a peak in one hour and slowly returned to the basal level 4 hours later.

Concomitant with the fall in blood inorganic phosphate

there .....

there is a diminution of phosphate excretion. Again a similar result can be obtained with insulin (Berlzveig, Lawhen and Keefer, 1929; Barrenscheen et al, 1927). Allan, Dickson and Markowitz (1924) pointed out that the initial decrease in phosphate excretion was followed by a pronounced increase in excretion, the total phosphates excreted in 24 hours being in excess of that excreted by uninjected normal animals. They stated that insulin and adrenaline were not antagonistic in effects on phosphate metabolism. Bolliger and Hartman (1925) also emphasized this lack of antagonism between insulin and adrenaline.

(111) <u>The adrenal cortex</u> Britton and Silvette (1937) maintain that adrenalectomised animals show hypoglycaemia and decreased liver and muscle glycogen stores independent of changes in electrolytic balance. Long and Katzin (1935) and Katzin and Long (1938) have recently shown that injection of adrenal cortex extract restored the glycogen stores of fasted hypophysectomised rats. Long and Lukens (1936) and Long, Fry and Thompson (1936) have shown that pancreatic diabetes was ameliorated after adrenalectomy just as it was after hypophysectomy. This did not occur if the medulla only was removed. Implantation of adrenal cortical tissue into partially depancreatised rats produced a reappearance of glycosuria. Long (1937) and Britton and Silvette (1937) have reviewed this subject.

Since carbohydrate and phosphorus metabolism are so intimately

connected .....

connected and since the adrenal cortex affects carbohydrate metabolism, it might be expected to affect phosphorus metabolism. The effects of the adrenal cortex on the mineral constituents of blood has also frequently been quoted. It is interesting to recall the important role the adrenal cortex plays in the various phosphorylating processes. (Verzar and Laszt, 1936 a,b; Verzar and McDougall, 1936; Verzar, Hubner and Laszt, 1937; Isselhutz, Laszt and Verzar, 1936.)

(iV) Serum calcium. Conflicting results have been reported with injections of adrenaline and the effects are probably secondary to changes in inorganic phosphate. Increased excretion of calcium after adrenaline injection have been reported by Quest (1909), Schiff and Peiper (1920) and Elfer and Kappel (1920). A rise in blood calcium (Vollmer, 1923 a, b, c; Pulay and Richter, 1926; Jacobson and Rothschild, 1927; Horsters, 1930 and a slight fall (Leicher, 1922; Billingheimer, 1922; Hetenyi and van Gaal, 1931; Woringer, 1924) have been reported.

In the sequel the effect of adrenaline on the plasma inorganic phosphate of the South African clawed toad is described and discussed and it is interesting to compare the effects with those occurring in mammals.

(p) The Pituitary glands

A. The anterior lobe. A relationship between the pituitary

gland .....

gland, calcium and phosphorus metabolism is indicated by the well established effects of hypophysectomy and administration of anterior lobe substance on the growth and ossification of the bones in animals and by the striking skeletal changes in acromegaly and pituitary gigantism or dwarfism in man. That the anterior pituitary affects the level of the blood inorganic phosphate has been suggested by several workers (Houssay and Mazzocco, 1922; and Marenzi and Gershman, 1934.) This effect can be produced by influencing the absorption, intermediary metabolism or excretion of phosphorus. Up to the present no effect on the absorption has been described. Phosphorus is absorbed very readily most of the phosphates being freely soluble both in water and in lipides as will be discussed in the sequel.

Possible mechanisms whereby the level of the blood inorganic phosphate may be affected are tabulated below:-

(1) An effect on the plasma constituents,

(2) An indirect following changes in carbohydrate metabolism (possible fat and protein metabolism as well).

(3) An indirect effect following stimulation of some other gland or glands.

(4) A direct effect on the renal threshold for phosphate.

(1) <u>An Effect on the Plasma constituents.</u>

Numerous papers from Houssay and his co-workers have been published, showing the effects of the anterior

pituitary .....

pituitary on the mineral constituents of the blood. Gerschman (1921), Houssay and Mazzocco (1922); Mazzocco (1927) and Gerschman (1931) found no change in serum calcium of dogs after hypophysectomy but a fall in blood inorganic phosphate. Changes in other mineral constituents also occurred. Marenzi and Gerschman (1934) found an insignificant diminution of blood inorganic phosphate of hypophysectomised dogs though other mineral changes were observed. Kobayashi (1931), Ichigo (1934), Cannavo and Beninato (1934) and Cannavo (1935) reported a fall in blood inorganic phosphate after hypophysectomy. Anderson and Castler (1938) found no effect on the calcium but the plasma inorganic phosphate was markedly lowered in hypophysectomised rats. Charles (1931) showed that the removal of the anterior lobe alone or both lobes in Xenopus laevis produced a persistent fall in serum calcium of 37 per cent and 26 per cent respectively. Hogben, Charles and Slome, (1931) found that the fall in serum calcium after hypophysectomy was identical throughout the year and was this independant of seasonal variations. They found that "both lobes exercise some influence direct or indirect on calcium metabolism, since the fall in calcium content following on removal of the anterior lobe alone is greater than the fall consequent on the removal of both lobes." This was subsequently confirmed by Shapiro and Zwarenstein (1933).

It .....

It will be noted that the calcium changes after hypophysectomy have been investigated in the clawed toad but no experiments on blood inorganic phosphate have hitherto been recorded. In the sequel experiments on plasma inorganic phosphate and the correlation with calcium are described.

Injections of alkaline anterior pituitary extracts (Marenzi and Gerschman, 1935) produced a hyperglycaemia and a rise in calcium and inorganic phosphate. Other mineral changes also occurred. Gerschman and Marenzi (1935a) found that with alkaline extracts the rise in calcium and inorganic phosphate occurred in thyroidectomised but in thyroparathyrodectomised dogs this was less well marked. Friedgood and McLean (1937) observed that repeated alkaline extracts of anterior pituitary produced a rise in calcium but no change in inorganic phosphate in guinea-pigs. Cannavo (1932) showed that injections of "Prolan" into normal and diseased human subjects produced no change in serum calcium nor in inorganic phosphate. In dogs and rabbits there was a definite rise in inorganic phosphate but no change in calcium occurred. Shapiro (1934) found an increase in serum calcium of cats injected with anterior pituitary extract.

The bulk of evidence indicates that blood inorganic phosphate is subject to changes after hypophysectomy and anterior pituitary administration. Hypophysectomy leads

to a .....

to a fall in inorganic phosphate, serum calcium remains unaffected or shows a drop, injection of anterior pituitary extracts produces a rise in inorganic phosphate and usually a rise in calcium. It will be noted that in this case calcium and inorganic phosphate do not react inversely to each other.

(2) Carbohydrate, Fat and Protein metabolism. It is well known that the anterior pituitary has a profound effect on carbohydrate metabolism. This subject has recently been re-viewed by Young (1938c, 1939) and Russell (1936). The great hypersensitivity of hypophysectomised animals to insulin (Houssay and Magenta, 1925) and the amelioration of pancreatic diabetes by hypophysectomy, and the long survival of animals so operated (Houssay and Biasotti, 1930) are of fundamental importance. The former will be further discussed in the sequel.

Effects of hypophysectomy on carbohydrate metabolism. Well fed hypohysectomised animals show no abnormality in carbohydrate metabolism except in response to insulin and adrenaline. It is generally accepted that fasting is the essential factor in producing hypoglycaemia. Normal blood sugar levels and carbohydrate reserves are the rule in well-fed animals but quite low values have been reported in some animals (Russell, 1936). This low carbohydrate store can be raised by anterior lobe therapy (Houssay et al, 1933).

Effect of anterior pituitary extracts. When suitable extracts are administered to normal animals the result is a high fasting blood sugar, glycosuria, raised sugar tolerance curve, failure of the respiratory quotient to rise on feeding carbohydrate, hyperlipaemia and ketonuria (Newton, 1939). Several separate factors have been differentiated for example glycotrophic, blood sugar raising, ketogenic, pancreatrophic and thyrotrophic factors.

Effect of pancreatectomy and hypophysectomy. That hypophysectomy immediately relieves the diabetic symptoms of pancreatectomised animals has been found in a large number of species including catfish, toads, rats, cats, dogs and monkeys (Russell, 1938). The blood glucose of these "Houssay" animals is more or less normal and there is very little glycosuria or ketonuria. The essential metabolic disturbance produced by pancreatectomy, however, is not obviated by hypophysectomy. The animal hovers between hypoglycaemia and hyperglycaemia according to its diet.

Since the metabolism of carbohydrate and of inorganic phosphorus are so intimately connected it might be expected that changes in carbohydrate metabolism would be reflected by changes in phosphorus metabolism. In more complex animals this may be masked and so by using a relatively simple animal the effects of anterior pituitary on inorganic phosphate may be made manifest. In the sequel the effect of

the pituitary on the plasma inorganic phosphate of Xenopus laevis will be discussed.

The effect of the anterior pituitary is complex, as it acts directly on carbohydrate metabolism and indirectly through the other endocrine glands. Thus it would not be surprising if the effects of hypophysectomy and anterior pituitary injection were very variable. Extracts of anterior pituitary contain many principles. For example if an extract rich in glycotrophic principle (Young, 1938b) were injected the peripheral action of insulin would be inhibited. Since insulin produces a fall in inorganic phosphate this action would be inhibited by the extract and the plasma inorganic phosphate might consequently rise. Again, injection of anterior pituitary extract rich in blood sugar raising principles produces a hyperglycaemia, in fact continuous injection may lead to a persistent diabetes (Young, 1937, 1938a). The hyperglycaemia may at first reflexly elicit a secretion of insulin which may produce a fall in blood inorganic phosphate.

Recently International Standards for some of the anterior pituitary hormones have been made and various standardised preparations are available at the present time. By the use of standardised extracts problems such as the mode of action of the anterior pituitary on phosphorus metabolism may be solved.

That the anterior pituitary has an effect on fat and protein metabolism is well known (Russell, 1936). Howphosphorus

is .....

is involved in the metabolism of fat and protein has already been indicated briefly. An effect on plasma inorganic phosphate may thus theoretically be due to involvement of fat and protein metabolism.

(3) Stimulation of other Endocrine glands.

(a) The adrenal cortex. The adrenotrophic principle of the anterior pituitary may play a part in carbohydrate and consequently in phosphorus metabolism. The pituitary has a well known effect on cortical function. Smith (1930), Evans (1933) demonstrated atrophy of the adrenal cortex after hypophysectomy or hypertrophy after injection of active extracts of anterior pituitary. Houssay and Sammartino (1933a) have shown that hypophysectomy produces atrophy in the zona fasciculata and zona reticularis whereas the zona glomerulosa hypertrophies.

Whether the anterior pituitary acts on carbohydrate metabolism directly (Houssay, 1937) or whether it does so through the adrenal cortex (Corey and Britton, 1939) it will eventually affect phosphorus metabolism.

(b) The gonads. The pituitary has a well marked effect on the gonads. Removal of the anterior pituitary leads to retardation of sexual development and genital atrophy (Cameron, 1936) Anterior pituitary implants (Smith and Engle, 1926a,b; Zondek and Aschheim, 1927a,b;) markedly accelerate sexual maturity. Shapiro and Shapiro (1934) have confirmed

the .....

the pituitary-gonad relationship in Xenopus laevis.

The effect of the ovary on phosphorus metabolism has already been discussed. The pituitary may affect the blood inorganic phosphate through the gonads. In the South African clawed toad, the pituitary and the gonads produce marked effects on serum calcium (Shapiro and Zwarenstein, 1933). Both the pituitary and the gonads control calcium metabolism and although the pituitary stimulates ovarian activity the action on blood calcium is not through the ovaries. It would be interesting to note the effect of the pituitary and gonads on plasma inorganic phosphate and this subject is discussed in the sequel.

Injection of gonadotrophic hormones in pigeons produced a rise in serum calcium in normal, hypophysectomised and thyroidectomised but not in castrated birds (Riddle and Dotti, 1936). A rise in blood inorganic phosphate in fowls injected with gonadotrophic hormones has been described by Laskowski (1938).

(c) Thyroid. The relationship between the pituitary and thyroid has been reviewed by Thomson and Collip (1933). It has been well established that active extracts of anterior pituitary stimulate the thyroid with the production of thyroid secretion. Hypophysectomy leads to atrophy of the thyroid gland.

The effect of the thyroid gland on phosphorus metabolism has already been discussed. Changes in blood inorganic phosphate

produced .....

produced by the anterior lobe of the pituitary may be due to secondary stimulation of the thyroid gland.

(d) The parathyroids. The existence of a parathyrotrophic hormone has not yet been definitely established. Houssay and Sammartino (1933b) produced atrophy or degenerative lesions in the parathyroid glands of the majority of dogs after hypophysectomy. Changes also occurred in depancreatised dogs but less frequently and less intensely. The frequency and intensity was maximum in "Houssay" animals. Anselmino, Hoffmann and Herold (1933) have found that injection of anterior pituitary extracts in male rats produced enlargement of the parathyroids. Friedgood and McClean (1937) reported that their extracts exhibited parathyrotrophic activity. Anderson and Oastler (1938) were unable to demonstrate a parathyrotrophic effect of the anterior pituitary in their experiments on rats.

Changes in blood inorganic phosphate produced by the parathyroids have been discussed. The effect of the anterior pituitary may be due to secondary stimulation of the parathyroids. Gerschman and Marenzi (1935a) found that injection of anterior pituitary extract into normal animals produced a rise in inorganic phosphate and calcium. The same effect was observed in thyroidectomised animals. In thyroparathyroidectomised animals, however, there was practically no change in calcium and inorganic phosphate. They concluded that the action of the anterior pituitary extract on the plasma constituents was indirectly through the parathyroids.

Gerschman .....

Gerschman and Marenzi (1935b) also showed a rise in inorganic phosphate and a fall in calcium following hypophysectomy and following pancreatectomy. Both produced lesions in the parathyroids (Houssay and Sammartino, 1935) and they attributed the changes in blood inorganic phosphate to hypoparathyroidism. Removal of the pancreas alone, however, produced a greater rise than removal of the pancreas and anterior pituitary.

(e) The pancreas. Anselmino and Hoffmann (1933) have reported that anterior pituitary extracts caused enlargement and increase in the number of islets of Langerhans and a slight fall in blood sugar. The effect of insulin on the blood inorganic phosphate has already been discussed. It is possible for the anterior pituitary to act on blood inorganic phosphate by means of a pancreatrophic principle.

(4) Effect on the renal threshold for phosphorus.

Brull and Eichholtz (1925) pointed out that the isolated kidney of Starling and Verney (1925) was unable to excrete inorganic phosphate and this was not due to lack of phosphatase (Eichholtz, Robison and Brull, 1925). They postulated that it was due to absence of pituitary or third ventricular influence. Removal of the pituitary or influence of the tuber cinereum in the intact animal abolished the normal secretion of inorganic phosphate although extracts of anterior pituitary were unable to restore the secretion.

Posterior lobe extract had irregular effects. This effect was not due to the polyuria produced by removal of the posterior lobe and the kidney retained its ability to excrete urea, hydrolyse organic phosphates and excrete inorganic phosphate when the threshold was reached. Brull (1937) showed that the renal threshold for phosphate was raised immediately after hypophysectomy. This produced a rise in plasma inorganic phosphate and a fall in urinary phosphate. This renal threshold for phosphate was lowered by injection of parathyroidhormone. Gonadotrophic, thyrotrophic, pregnancy urine, and posterior lobe extracts failed to restore the excretion of phosphate. These experiments were performed on dogs under narcosis and refer to events shortly after hypophysectomy.

B <u>The posterior lobe</u>

That the posterior lobe may be involved in metabolism of fat and protein has often been suggested (Best and Taylor, 1937) Geiling and DeLawder (1932) have shown that after the injection of posterior pituitary extracts the muscles for a short time pass into a state suggesting that their activity was being carried out anaerobically. The blood issuing from the muscles had a high inorganic phosphate content. This phase was followed by one in which the conditions were reversed.

Burn (1923) showed that subcutaneous injections of extracts

of the ....

of the posterior lobe of the pituitary given simultaneously with injections of insulin, diminished or abolished the fall in blood sugar produced by insulin. Geiling, Campbell and Ishikawa (1927) found that "posterior pituitary extract" protected hypophysectomised dogs against insulin hypoglycaemia.

The effect of the posterior lobe on the serum calcium of Xenopus laevis has already been discussed. In the sequel experiments showing the effect of the posterior lobe on plasma inorganic phosphate are described.

2. FACTORS INFLUENCING THE ABSORPTION OF PHOSPHORUS FROM THE ALIMENTARY CANAL.

The chief factors determining cellular absorption are the fat or water solubility of the substances concerned. Many of the phosphorus compounds are miscible both with water and with fat and thus are easily absorbed. Most of the inorganic phosphate salts are readily soluble in water and intestinal absorption is easy and rapid. Organic phosphorus compounds such as phospholipides and nucleotides are hydrolysed, inorganic phosphate finally being released and rapidly absorbed. For example, lecithin is broken down to fatty acids, choline and glycerophosphoric acid (Bokay, 1877). The glycerophosphoric acid is split up by the phosphatase of the intestine to glycerol and phosphoric acid (Grosser and Husler, 1912) both of which are readily absorbed.

Phosphorus is of great importance in the absorption of

calcium ....

calcium. Calcium is only absorbed if it is in a water soluble form (Verzar and McDougall, 1936). The reaction of the intestine is of great importance, substances increasing the acidity of the intestinal contents increase the absorption of calcium and vice versa. If phosphates are present in large enough quantities insoluble calcium phosphates are produced and their absorption is thus decreased. (Orr et al., 1924)

Vitamin D increases the amount of calcium and phosphorus retained in the body on any given diet. The rachitic infant stores much less calcium and phosphorus in his body than does a normal child and administration of vitamin D produces marked improvement in the normal balance (Clouse, 1932). Varkany (1932) has shown that feeding inorganic phosphate resulted in a temporary rise in inorganic phosphate of the blood. In fasting normal rabbits the increase was surprisingly constant. When they were given large doses of irradiated ergosterol before the phosphate was fed the average rise in blood inorganic phosphate was doubled or trebled. Non-rachitic babies also showed a constant increase in blood inorganic phosphate with equivalent doses of sodium phosphate. Rachitic babies, however, showed no increase in blood inorganic phosphate until anti-rachitic treatment was begun, when the phosphataemic reaction rapidly became normal or greater than normal.

Nicolaysen (1937) in an extensive series of experiments

found .....

found that vitamin D had no influence on the absorption of phosphate from the gut. In calcium starvation the absorption of inorganic phosphates and glycerophosphates was the same whether vitamin D was present or not. A reduced absorption of phosphate in vitamin D deficiency was due to precipitation with unabsorbed calcium in the gut. Calcium absorption was decreased in the absence of vitamin D whereas that of phosphate proceeded at the same rate irrespective of the presence of the vitamin.

Schmidt and Greenberg (1935) have reviewed the literature on the absorption and excretion of calcium and phosphorus.

Recently the absorption of phosphorus has been intensively studied by means of radio-phosphorus. Chievitz and Hevesy (1937) by adding radio-phosphorus to the diet, demonstrated that an appreciable part of the phosphorus found its way into the bones, teeth, muscles and other organs of both growing and adult animals. When sodium phosphate was injected the labelled phosphorus rapidly disappeared from the circulation. This was thought to be due to absorption of the phosphate ions by the calcium phosphate of the skeleton. Hahn, Hevesy and Lundsgaard (1937) have calculated that the average time which a phosphorus atom spends in the body is 30 days. Cohn and Greenberg (1938) administered sodium hydrogen phosphate orally to adult rats.

They .....

They found that the major deposition of the phosphate occurred within the first 8 hours after administration. Absorption of orally administered phosphate to animals fasted for 24 hours was most rapid in the first two hours and was usually at an end in 6 hours. About 30 to 40 per cent of the phosphate passed through into the faeces unabsorbed.

Dole et al., (1937, 1938a,b;) found that there was no significant difference between the absorption of labelled phosphorus in normal and in rachitic animals.

3. FACTORS INFLUENCING THE EXCRETION OF PHOSPHORUS.

The main routes of excretion of phosphorus are through the kidneys and the intestine. Through the former the major part is excreted. Phosphates are readily absorbed and rapidly excreted in the urine. (Cushny, 1918). Phosphate excretion is grossly influenced by a number of factors (Smith, 1937) particularly by changes in acid-base equilibrium. The normal threshold of the kidney can be readily altered for example in acidosis phosphate excretion is markedly increased although the plasma level may be below normal.. The effect of the parathyroid and the anterior pituitary on the renal threshold have already been discussed. The ability of the kidney to hydrolyse organic phosphorus compounds is well known.

Chievitz and Hevesy (1937) showed that the salts of phosphorus administered by mouth or parenterally, are excreted both by the intestines and by the kidney. In man

it was calculated that about 1/7 of the faecal radio phosphorus came from excreted materials entering the intestine through the digestive juices. About 12 per cent of the phosphorus administered with the food appeared in the urine of human subjects within 24 hours and after this only 2 to 3 per cent per day. An adult rat excreted phosphorus about equally in the faeces and in the urine 25 per cent being eliminated in the first 3 days. Cohn and Greenberg (1938) in rats, found that 30 to 40 per cent of administered radiophosphorus was unabsorbed, 20 to 30 per cent excreted in the urine within 6 hours and about 3 per cent found its way back into the intestines.

The factors influencing phosphorus excretion may conveniently be summarised as follows:-

(a) Ductless glands

(b) Nature of the diet

(c) Vitamin D.

(a) Ductless glands. The effect of the parathyroids, thyroid, gonads, pancreas, adrenals and pituitary on the excretion of calcium and phosphorus has already been discussed.

(b) Nature of the diet. Shohl and Bennett (1928), Shelling (1932) and Shelling and Asher (1932) showed the importance of measuring and controlling both the calcium and the phosphorus and the relative amounts of these two elements in the diet when investigating calcium and phosphorus metabolism. Robinson, Huffman and Mason (1929) demonstrated that serum

calcium .....

calcium was raised by calcium chloride and by calcium lactate plus lactose. Blood inorganic phosphate was increased by bone meal, lactose or by bone meal plus lactose. It was reduced by calcium lactate or by calcium lactate plus lactose. The administration of calcium chloride led to a marked loss of phosphorus exceeding that present in the diet. This loss was distributed between the urine and the faeces. Lactose with bone meal doubled the calcium and phosphorus retention. These experiments show that diet, particularly the amounts of calcium and phosphate present, affect the blood calcium and phosphate and the amounts of these elements excreted.

In the sequel it will be shown that the South African clawed toad is eminently suitable for experiments on phosphate metabolism. When the animal is first brought into the laboratory (during the anoestrous period) the plasma inorganic phosphate value is higher than after it has been in the laboratory for a few days. Once the value has fallen, however, it remains very constant and there is little change for several months under laboratory conditions. Shapiro (1933b) found that serum calcium displayed a remarkably narrow range of variations, captivity affect only appearing after 3 - 4 months.

(c) Vitamin D. Pugsley (1932) working with irradiated ergosterol in rats, found a very marked increase in urine calcium

but .....

but a decrease in faecal calcium and phosphorus after oral administration of 20,000 - 50,000 international units daily. Nosignificant change in urinary phosphate occurred. Jones and Robson (1933) produced soft fragile bones with continuous administration of irradiated ergosterol. The calcium and phosphorus liberated into the blood stream must have been excreted via the kidneys as the faecal route remains unaffected or even depressed by the vitamin. It is important, however, to distinguish between the effects of normal and toxic doses. Vitamin D is chiefly concerned with the intermediary metabolism of calcium and phosphorus and probably normally has little effect on the excretion of these elements.

Dole et al., (1937, 1938a,b;) found that the excretion of labelled phosphorus (radio-phosphorus) was the same in rachitic as in normal animals.

- EXPERIMENTAL -

1. OBJECT OF INVESTIGATION

The object of this investigation is to determine the influence of the endocrine organs on the plasma inorganic phosphate of an animal low down in the developmental scale. Most of the work hitherto published has been performed on mammals and it was thought that by working with simpler animals, whose compensating mechanisms and reactions might be less complex and less well-developed, the effects of gland removal and injection of active extracts would be made manifest.

Shapiro (1933b) has shown the effect of the pituitary gonad relationship on serum calcium in Xenopus laevis and it was considered that it would be of great interest to investigate the relationship to inorganic phosphate and to correlate changes in serum calcium with those of plasma inorganic phosphate. Seasonal variations in inorganic phosphate have been worked out and compared with the known changes occurring in serum calcium.

Furthermore the clawed toad was chosen because of the ease and completeness with which anterior lobe hypophysectomy can be performed. An exact index of the completeness of the operation can be gained by using the colour response, as will be described and this obviates the long and tedious procedure of cutting serial sections through the brain. Wherever possible the inter-relationships between the various endocrine glands and the anterior pituitary in respect of

their effects .....

their effects on plasma inorganic phosphate have been investigated.

(2) METHOD OF COLLECTION AND STORAGE OF ANIMALS.

The toads were collected from ponds in the Cape Peninsula near Mowbray and brought into the laboratory. There they were placed in large metal tanks 36 by 18 inches in size, there being 2 inches of water in the tanks. Overcrowding with animals was avoided, not more than 6 dozen toads being kept in a tank at one time. When fresh animals were sent in they were kept in the laboratory for several days before being used for injection or subjected to operative procedures. During this period they usually evacuated large amounts of shell-fish and other food from their natural habitat. The only exception to this procedure was in the conduct of seasonal variations the animals being killed on the same or following day after being brought into the laboratory.

During all experiments extending over several months, the animals were fed at regular intervals twice weekly with raw minced meat and the water in the tanks was changed daily except on Sundays. Animals fresh from the pond were never fed.

(3) METHOD OF COLLECTION OF BLOOD FOR INORGANIC PHOSPHATE ESTIMATIONS.

The bulk of the work was carried out on female toads as they are larger and yield more blood.

The .....

The pithed toad is laid on its back on a marble slab and the pectoral skin removed. With a pair of sharp scissors the xiphisternum is cut through and the cartilages severed at their junction with the humeri avoiding the large subclavian vessels. The triangular flap thus marked out is removed and the heart exposed lying in its pericardial sac. The sac is incised and the heart exposed, beating in situ. All the body lymphatic and pericardial fluids are absorbed with filter paper and the ventricle cut, while the animal is held with the heart suspended over a waxed dish containing a few grains of heparin. Occasionally the toads may have a trematode infection in the pericardial sac. If so the trematodes are removed by careful drying with filter paper.

Collection of blood in this way prevented clotting and care was taken to avoid expressing body fluids into the blood. At first the dishes were kept in ice and the toads bled into the dishes thus frozen, but this was found to be unnecessary as heparinised blood did not clot at room temperature. The number of toads used for each sample of blood varied but on an average two were sufficient. The blood was transferred to a centrifuge tube (waxing of which was necessary) and centrifuged at the rate of 3,500 revolutions per minute for 4 minutes. No laking occurred at this speed. The plasma was then pipetted off and used for the chemical estimation. The number of toads used for each estimation varies and is stated in every table.

During .....

During the investigation approximately 6,000 toads were used and 2,750 estimations performed.

Choice of an anticoagulant.

In the absence of an anticoagulant freezing of the blood did not prevent clotting of the plasma in the centrifuge tubes even when the latter were waxed.

Potassium oxalate and sodium citrate were then tried out as anticoagulants but difficulties with clotting of the plasma and with colorimetric determinations were encountered and ultimately it was found that heparin was the most satisfactory anticoagulant. The inorganic phosphate content of heparin was estimated and found to be negligible. Gaebler (1932) has reported that heparin does not affect the plasma inorganic phosphate, serum and plasma inorganic phosphate being of the same order of magnitude. Some preparations of heparin, however, contain materials transmitted to the trichloracetic acid and filtrate during the inorganic phosphate determinations causing a slight turbidity in the final solution. This difficulty was not encountered with amphibian blood.

As no heparin could at first be obtained commercially, heparin was prepared by the method of Charles and Scott (1933). Later Hinson, Westcott and Dunning and B.D.H preparations were obtained.

Preparation of heparin

Ten pounds of fresh beef liver was minced and allowed

to .....

to stand at room temperature (20°C) for 24 hours. The tissue was then extracted with 6.2 litres of 0.5 N sodium hydroxide and 0.75 litre saturated ammonium sulphate solution. The mixture was slowly heated with constant stirring to 45°C in a steam jacketed vessel and maintained at this temperature for one and one half hours. The mixture was then rapidly heated to 70°C to coagulate the proteins. It was next filtered while hot through Buchner funnels and glass wool. The dark-brown filtrate was acidified to pH 2 (using trapeolin 00 as indicator) with sulphuric acid. This precipitated most of the heparin. The acid mixture was heated to 60°C and filtered through linen cloths while still hot and the precipitate washed with 2 litres of hot dilute sulphuric acid pH 2 - 2.5. This removed traces of ammonium sulphate. The precipitate obtained was suspended in 0.6 litre 95 per cent ethyl alcohol for 20 hours, extracting a brown oily substance.

The alcohol was decanted and the precipitate centrifuged and dissolved in 0.6 litre water. The reaction was adjusted to pH 6 (phenol phthalein as indicator). Two point 5 gramme trypsin in aqueous suspension and 1.0 ml xylol were added to the solution. This mixture was placed in an incubator at 36°C for 36 hours. Every 12 hours the solution was readjusted to pH 6.

After digestion 1.2 litres of 95 per cent ethyl

alcohol .....

alcohol were added and the mixture made slightly acid with hydrochloric acid. After standing for 24 hours the precipitate settled quite firmly to the bottom of the flask leaving the supernatant fluid free from heparin. The precipitate was removed and added to 150 ml. water containing just enough 0.5N sodium hydroxide to dissolve it. The solution was heated to 75°C to destroy the trypsin present and after cooling was centrifuged to remove insoluble material. The anticoagulant was precipitated by adding two volumes of acetone and the mixture made slightly acid to litmus with hydrochloric acid. The suspension was allowed to stand for 15 hours, then the precipitate was centrifuged and washed with 95 per cent ethyl alcohol and dried at room temperature. It weighed about 0.5 gramme.

(4) METHOD OF ESTIMATION OF INORGANIC PHOSPHATE.

At first estimations were carried out using whole blood and estimating for inorganic phosphate by the method of Brigge (1922, 1924), but unsatisfactory results were obtained. Youngburg and Youngburg's method (1930), using plasma, was then adopted and was found to give satisfactory results.

In this method the blood proteins are precipitated with trichloracetic acid and the filtrate used. The filtrate is treated with a molybdic-sulphuric acid reagent forming a phosphomolybdate. Stannous chloride is added, according to the method of Kuttner and Cohen (1927) and Kuttner and Lichtenstein (1930), to reduce the phosphomolybdate selectively,

with .....

with the production of a blue colour due to the colloidal reduced oxides of molybdenum. The amount of phosphate is estimated colorimetrically against a standard phosphate solution of known concentration. In the Bell and Doisy method (1920) the reduction is carried out with hydroquinone and by adding an acid solution. Briggs,(1922, 1924) stabilised the colour. Fiske and Subbarow (1925) used aminonaphthol-sulphonic acid.

With the Youngburg and Youngburg method (1930) it was found that the colour developed very quickly and the final solution obtained remained stable for some time. Several standards were always made up and matched against each other before estimating the plasma inorganic phosphate thus eliminating any error from this source. Whenever fresh standard phosphate solution was made up, samples were matched with the discarded solutions. It was found that chloroform preserved the solutions adequately and in no case was there any deterioration of the solutions.

Particular care must be taken to add the stannous chloride to the molybdic acid reagent and trichloracetic acid filtrate, without delay, to prevent deterioration of the colour. Stannous chloride solutions were made up fresh each day, as it was found that the solutions were rapidly oxidised. The stock solution was changed every two months and stored in a cool place in a dark brown bottle.

Amphibian whole blood in contrast to human blood was unsuitable as the final solution was turbid and centrifugalisation

not .....

not only removed the turbidity but also some of the coloured compound, which was adsorbed onto the deposit. The use of plasma obviated this difficulty.

Great care must be taken to avoid laking; as this produced an increased phosphate value. If the plasma was tinged with laked blood it was discarded. Plasma filtrates were found to be stable and could be kept for several hours without change in inorganic phosphate content. Gaebler (1932) has reported similar results and states that whole blood filtrates, on the other hand, cannot stand as organic phosphorus compounds (which are found chiefly in the red blood cells) are rapidly converted to the inorganic form. Sahyun (1933) has reported similar changes in whole blood.

IN EVERY TABLE, UNLESS STATED TO THE CONTRARY, ALL FIGURES ARE EXPRESSED AS mg.P/100 ml. PLASMA. THE FIGURES IN BRACKETS REFER TO THE NUMBER OF ESTIMATIONS. THE STANDARD ERROR OF EACH SERIES OF ESTIMATIONS IS PUT BESIDE THE AVERAGE INORGANIC P VALUE.

(Standard error is calculated from the formula $\frac{\Sigma d^2}{n(n-1)}$ (Burn, 1937) where Σd^2 is the sum of squares of the deviations from the mean and n is the number of estimations).

(5) OPERATIVE PROCEDURES.

(a) Anaesthesia.

Animals .....

Animals are placed in a small rectangular glass dish, 6 inches deep, containing a mixture of ether and water (1:5). An immediate excretion of thick mucus occurs and the animals become very active. When the voluntary movements have ceased the animals are removed and laid on their backs. An adequate depth of anaesthesia is obtained when the animal is unable to recover from the supine position. Light anaesthesia, i.e. when the animal twitches although on its back, is quite sufficient for hypophysectomy as the animal remains supine for about 5 minutes.
For pancreatectomy and thyroidectomy the animal must be fairly deep, remaining anaesthetised for about 20 minutes. These animals are not removed from the ether until all movements have ceased and the animals lie completely flaccid. It is largely a matter of experience to gauge the exact depth of anaesthesia.

Commercial "Natal" ether can be used for anaesthesia, but occasionally samples are very toxic. It was found most suitable to use purified "Anaesthetic" ether.

(b) Post-operative care.

After hypophysectomy the animal is laid in a tray containing water about half an inch deep, until it has recovered from the anaesthetic. It is then transferred to a sink and left for about 12 hours. The water in the sink is kept running but does not accumulate as the plug is removed. After about 12 hours the animals can be transferred

to .....

to tanks and kept there in the usual way. Anterior lobe hypophysectomised animals are kept in tanks painted white on the inside and completely hypophysectomised animals in tanks painted black on the inside.

With abdominal operations, however, post operative treatment is different and is of vital importance. As soon as the animals recover from the anaesthetic they should be put in water about 2 inches deep. If this is not done and animals are left in a sink, they stick to the floor of the sink, and a high mortality rate (ca 50 per cent) results. Berk (1939a) and Gitlin (1939b) have found similar results. Shapiro (1933b) found these precautions unnecessary.

(e) HYPOPHYSECTOMY

The operative procedure was according to the method of Hogben (1923). Animals were lightly anaesthetised and placed on their backs. The mouth was held open by a forceps one blade of which was narrow and was held in one internal naris. The other blade was flattened out and kept the lower jaw retracted.

A small hole was drilled in the roof of the mouth with a dental burr, through the mucous membrane and bone just in front of the opening of the eustacian tube. The drilling was continued until the anterior lobe of the pituitary could be seen shining through the remaining thin plate of cartilage. This was reflected with a thin pointed knife and the pituitary

was removed by gentle suction through a glass tube of 1.5 mm. bore attached to a water pump. By regulating the amount of suction the anterior lobe only or both anterior and posterior lobes could be removed.

The pituitary in Xenopus consists of a large anterior lobe (which is a tached to the tuber cinereum by the pars tuberalis) and a small posterior lobe (Hogben and Slome, 1931; Rimer, 1932). When the anterior lobe is removed the pars tuberalis is more or less completely removed with it. The tip, however, is adherent to the tuber cinereum and does not always completely come away with the anterior lobe. The pars tuberalis may regenerate. To avoid confusion it is convenient to refer to the anterior lobe as both pars anterior and pars tuberalis. Anterior lobe hypophysectomy thus refers to removal of pars anterior and pars tuberalis.

Only those animals which showed a maximum expansion of melanophores i.e. which remained permanently dark on a white background (light scattering surface) were retained for all experiments on anterior lobe hypophysectomised animals. After several months the white background response reappeared to a varying degree. Hogben and Slome (1931) attribute this to the regeneration of the pars tuberalis. The completely hypophysectomised animals remained pale on a black background (light absorbing surface) and the

colour .....

colour response never reappeared.

Operative controls were subjected to the same anaesthesia as the hypophysectomised animals and the same procedure of operation was adopted. All stages up to but not including the removal of the pituitary were carried out.

(d) PANCREATECTOMY.

The following technique for pancreatectomy was adopted. Animals were anaesthetised fairly deeply and laid on their backs on the operating table. The skin was incised in the midline for about one inch. A left paramedian incision of about an inch was made through the abdominal muscles and peritoneum extending up to the xiphisternum. A paramedian incision avoided the ventral abdominal vein which runs in the midline closely applied to the inner surface of the ventral abdominal wall. An eye retractor was inserted and the stomach and small intestine withdrawn through the incision. The pancreas was brought into view, a yellowish organ lying in the mesentery between the stomach and small intestine. With an electric or heated metal cautery the whole pancreas was removed taking care to avoid the mesenteric vessels and the bile duct. Occasionally it was found impossible to save the latter.

The abdominal wall was sutured with a continuous suture

of .....

of silk. The skin was sutured with interrupted cotton stitches.

Operative controls were treated in the same way as pancreatectomised animals except that one fat body attached to the upper pole of one ovary, instead of the pancreas, was removed.

(e) THYROIDECTOMY.

The topography of the thyroid glands in Xenopus laevis has been described by Rimer (1931). Thyroidectomy was performed according to the method described by Gitlin (1939a).

Animals were anaesthetised fairly deeply and laid on their backs. The skin and underlying sternum were cut through in the midline. A small retractor was inserted to keep the two halves of the sternum apart. The thin, transparent, non-vascular membrane connecting the sternohyoid muscles of the two sides was divided in the mid-line. Separation of the sternohyoids exposed the thyroid glands, one on each side. These were excised by means of a heated metal cautery. Muscle, cartilage and skin sutures completed the operation. In tying the stitches through the cartilage care was taken to avoid overlapping of the two plates.

Operative controls were made, all stages up to but not including the removal of the thyroid glands being performed.. A small fragment of fatty tissue was removed in the vicinity of the glands.

(f) GONADECTOMY.

Gonadectomy ....

Gonadectomy was performed according to the method of Shapiro and Zwarenstein (1933). Fresh pond animals were removed during the breeding season when the ovaries were at their maximum weight, namely July (Shapiro, 1933b; Shapiro, 1939b; Bork, 1939a). The animals were lightly anaesthetised the ventral skin was divided by a one inch median incision and the ventral abdominal muscles divided by a paramedian incision half an inch long. This was done to avoid the ventral abdominal vein and also to prevent subsequent herniation of abdominal contents. One ovary was removed from the incision at a time, and the mesentery cut through as close to the renal attachment as possible using a heated metal cautery. Care was taken to avoid the blood vessels supplying the fat bodies as they run close to the upper pole of the ovary and to leave whatever fat body there was. Care was exercised to avoid touching the large veins on the ventral surface of the kidneys. The muscle was closed with a continuous cotton suture, avoiding the ventral abdominal vein. The skin was closed by interrupted cotton sutures.

Orchidectomy was performed along similar lines but it was found more convenient to hold the testes with a pair of forceps instead of with the fingers.

Operative controls were treated in the same way as gonadectomised animals. In the females one lobule of one ovary was removed. In the males portion of one fat body was removed.

In all operations on Xenopus laevis aseptic precautions are unnecessary .....

unnecessary.

(g) METHODS OF INJECTION.

(i) Intraperitoneal injection. The needle of the hypodermic syringe is pushed through the ventral skin of the animal in the region of one of its flanks and the needle is run a little way between the skin and abdominal wall before piercing the muscle and entering the peritoneal cavity. By this means a valve flap is formed preventing any escape of fluid injected. Care is taken to avoid pushing the needle into any abdominal organ or into the pelvis.

(ii) Intralymphatic injection. In the experiments described in the sequel only the ventral lymphatic route was employed. The hypodermic needle is passed through the muscles of the floor of the mouth, pushed a little way between skin and muscles and then the injection is made into the ventral lymph space.

(6) METHOD OF PREPARATION OF ENDOCRINE EXTRACTS.

Acid extracts of sheep anterior pituitaries were prepared according to the method of Bellerby (1929).

Sheep heads were obtained from the Maitland abattoirs which are situated half an hour by motor car from the laboratories. The heads were obtained from animals immediately after they had been slaughtered.

Immediately upon arrival at the laboratories the heads were sawed open, the brain moved aside and the pituitaries

removed ....

removed together with and enclosed in the dura mater lining, the sella turcica and the diaphragma sellae. The pituitaries were immediately placed in a clean dry dish which was surrounded by ice. The pituitaries were then carefully shelled out of their dural investment and the posterior lobes and infundibula were removed, care being taken to leave only anterior lobes. These anterior lobes were weighed and transferred to a mortar. Clean dry quartz sand was added and the pituitaries ground up until the sand was a fine powder and no large pieces of anterior lobe tissue were recognisable. The mash was then transferred to a small beaker and 1.0 per cent acetic acid was added, 1.5 ml. for each gramme of anterior lobe. The mixture was then well stirred and left overnight at room temperature in a cool place with the mouth of the beaker covered with filter paper.

The following morning the mixture was centrifuged and the supernatant fluid collected. By removing the deposit of anterior pituitary, stirring and recentrifuging, a small additional quantity of extract was obtained. The extract was then neutralised to litmus by adding drop by drop of 40 per cent solution of sodium hydroxide. At the neutral point a fine flocculation occurred. This was removed by recentrifuging. The volume of supernatant fluid was measured and diluted.

These extracts are designated in this work by the letters "S.A.P." e.g. S.A.P. 1A, S.A.P. 1B. The strengths of the

extracts ....

extracts are given in the appendix: protocol No. 1. The letters S.A.P. stand for sheep anterior pituitary; A represents extracts where 1.0 ml. was equivalent to 1 anterior lobe; B where 0.6 ml. was equivalent to 1 anterior lobe; the ciphers 1, 2 etc. refer to different extracts.

To control the anterior pituitary extract an extract of sheep's brain was always made. The same weight of brain tissue as anterior pituitary was treated in an identical manner and the final solution similarly diluted.

(b) The insulin used in this work was a commercial preparation of crystalline insulin (Burroughs Wellcome & Co.) containing 100 International Units in 5 ml. phials.

(c) The adrenaline used was in tablet form: "Tabloid" adrenaline (Burroughs Welcome and Co.) 0.001 gm. per tablet. The tablets were dissolved in frog Ringer solution and injected.

(d) The parathyroid hormone used was a commercial preparation "Paroidin" (Parke, Davis and Co.) containing 100 Hanson units/ml. in 5 ml. phials. One Hanson unit is one one-hundredth of the amount required to produce a 1 mg. rise in the serum calcium of 15 kilo dogs 24 hours after parathyroidectomy.

(e) The thyroxine used was a B.D.H. preparation thyroxine sodium 0.2 mg. / tablet. The tablets formed a turbid solution in frog Ringer solution and were injected

in .....

in this form. A preparation made by Ciba Ltd. containing 1 mg / tablet of thyroxine sodium was also used and was soluble in frog Ringer solution.

(f) Crystalline oestradiol benzoate and progesterone dissolved in oil were employed (Organon - Dimenformon and Progestin). In every ampoule 5 mgs. crystalline substance was dissolved in oil.

S U M M A R Y.

(1) The objects of this investigation have been outlined.

(2) A description of the following has been given.

a. The method of collection and the storage of the animals.

b. The collection of blood, the use of anticoagulants and the preparation of heparin.

c. The biochemical methods of estimation of plasma inorganic phosphate used in this investigation.

d. The method of anaesthesia and the post-operative care employed.

e. The technique of hypophysectomy, pancreatectomy, thyroidectomy and gonadectomy.

f. The methods of injection employed.

g. The preparation of anterior pituitary extract and description of the hormones used (insulin, adrenaline, parathyroid hormone, thyroxine oestradiol benzoate and progesterone).

A.

The effect of temperature on plasma inorganic phosphate.

In view of the fact that the investigation would proceed over an extended period, on a cold blooded animal, it was considered necessary to ascertain whether changes in temperature would have any effect on the plasma inorganic phosphate. Should any change manifest itself due allowance would have to be made.

Two batches of female toads freshly collected from the pond were evenly distributed into two tanks. One tank was placed in an electrically regulated refrigerator for two weeks at a temperature of approximately 6°C. The other was placed in a self-regulating thermostat at a temperature of approximately 23°C. Temperature readings were taken daily at the same hour and the water changed every second day. The animals were removed at various intervals up to a fortnight for the estimation of plasma inorganic phosphate.

The experiments were repeated and the relevant data are shown in the following table.

- TABLE 1. - .....

TABLE 1.

Showing the daily temperature in the refrigerator and in the thermostat.

DATE	Refrigerator temperature in °C	Thermostat temp. in °C
9.2.37		22°C
10.2.37	9°C	22.5°C
11.2.37	8.5°C	22.5°C
15.2.37	6°C	22.5°C
16.2.37	9°C	23°C
17.2.37	6°C	23°C
18.2.37	8°C	23°C
19.2.37	7°C	23.5°C
22.2.37	6°C	23°C
23.2.37	7°C	22.5°C
Average	8°C	Average 23°C

TABLE 11.

Plasma inorganic phosphate levels in female toads maintained at different temperatures for two weeks.

No. of days in tanks	Refrigerator	Thermostat
7	5.8 (2)	5.5 (4)
10	6.7 (3)	6.1 (4)
14	5.1 (3)	6.7 (4)

Six dozen toads were used for this experiment. Detailed figures of the results appear in the appendix, protocol No. 2. Each determination required approximately four toads.

Discussion

From the results it is clear that a range of 15°C (i.e. from 8°C to 23°C) does not affect the plasma inorganic phosphate of the clawed toad. In the above table it would appear that the plasma phosphate rises in the thermostat but this was not confirmed in a similar experiment. The normal range of plasma inorganic phosphate will be considered in the following chapter.

It is interesting to note that Shapiro (1933b) has shown that temperature has no effect on the serum calcium on Xenopus laevis in view of the well-known relationship between calcium and phosphorus.

B CHANGES.....

B CHANGES IN THE PLASMA INORGANIC PHOSPHATE OF THE SOUTH AFRICAN CLAWED TOAD ASSOCIATED WITH CAPTIVITY AND THE NORMAL REPRODUCTIVE CYCLE.

Shapiro (1933b) has shown that the serum calcium of female toads maintained in captivity was significantly lower than that of animals brought in fresh from the ponds. The ovaries of captive females were severely atrophied after 6 months in captivity and often presented the appearance of gelatinous masses in which individual ova could no longer be distinguished on naked eye examination. On the other hand, the ovaries of normal pond animals at the height of the breeding season were filled with large ova easily visible microscopically. A definite cycle in which ovarian changes occurred was established. The ovaries were largest and filled to distension with ova during the breeding season i.e. during late winter and early spring. They were lightest and smallest in the summer months.

In the following work changes in plasma inorganic phosphate occurring in animals kept in captivity as well as seasonal variations in fresh pond animals have been investigated. These results have been correlated with changes in the serum calcium level of animals kept in captivity and with seasonal variations in serum calcium and ovarian activity.

Effect of captivity.

About 15 dozen toads were collected from their natural habitat in March, 1936 and kept in a large tank in the laboratory. Animals were killed at monthly intervals for

12 months ....

12months and their plasma inorganic phosphate estimated.

In Table 111 below the effect of captivity on plasma inorganic phosphate and serum calcium are shown.

The serum calcium figures are quoted from Shapiro(1933b).

GRAPH 1.

PLASMA INORGANIC PHOSPHATE AND SERUM CALCIUM OF FEMALE ANIMALS DURING CAPTIVITY.
SERUM CALCIUM.
PLASMA INORGANIC PHOSPHATE.
MG. Ca/100 ML.
MG. P/100 ML.
TIME IN MONTHS.

TABLE 111.

Plasma inorganic phosphate and serum calcium during captivity.

Time in months	Serum calcium	Plasma inorganic P			Gonad Ratio
0	11.7 ± 0.1	7.6	±	0.24 (9)	
1	(9.2)				
2	10.5 ± 0.8	4.7	±	0.15 (7)	8.8
3	11.0 ± 1.0	4.5	±	0.13 (4)	6.7
4	11.3 ± 0.9	4.9	±	0.36 (7)	7.4
5	10.1 ± 0.8	4.7	±	0.04 (3)	6
6	9.0 ± 0.8	5.0	±	0.04 (4)	9.4
7		6.7	±	0.26 (6)	7.2
8	9.0 ± 0.5	5.9	±	0.63 (4)	9.2
9	8.3 ± 0.3	5.6	±	0.29 (4)	5.7
10		5.7	±	0.41 (3)	6.3
11	8.4 ± 0.3	6.4	±	0.39 (5)	6.6
12		6.1	±	0.63 (5)	7.0

Each inorganic phosphate estimation required approximately 2 animals. Detailed figures appear in the appendix: protocol No. 3.

The above data are graphically represented in Graph 1 below. From Table 111 and Graph 1 it is seen that a marked fall of plasma inorganic phosphate occurred two months after captivity. This experiment was repeated but shorter periods of captivity were investigated.

TABLE 1V.

TABLE IV.

Plasma inorganic phosphate during a few weeks of captivity.

Normal	7.2	(4)
3 Weeks Captivity	5.9	(6)
5 Weeks Captivity	5.3	(2)

Each estimation required approximately 2 toads. Detailed figures appear in the appendix: protocol No. 3.

Similar experiments have shown that the fall in plasma inorganic phosphate occurs within the first week of captivity and following this fall the level remains constant for long periods of time. Consequently in subsequent injection and operative experiments, animals were kept up to a week in captivity before being used.

Seasonal variations in males and females.

At least one dozen males and one dozen females (see protocol No. 4) were collected from the same pond every month from December, 1937 to November, 1938. Animals were killed on the same day if possible but when they arrived in the late afternoon they were killed on the following morning.

In Table V the plasma inorganic phosphate of male and female toads is recorded.

Table V.....

TABLE V.

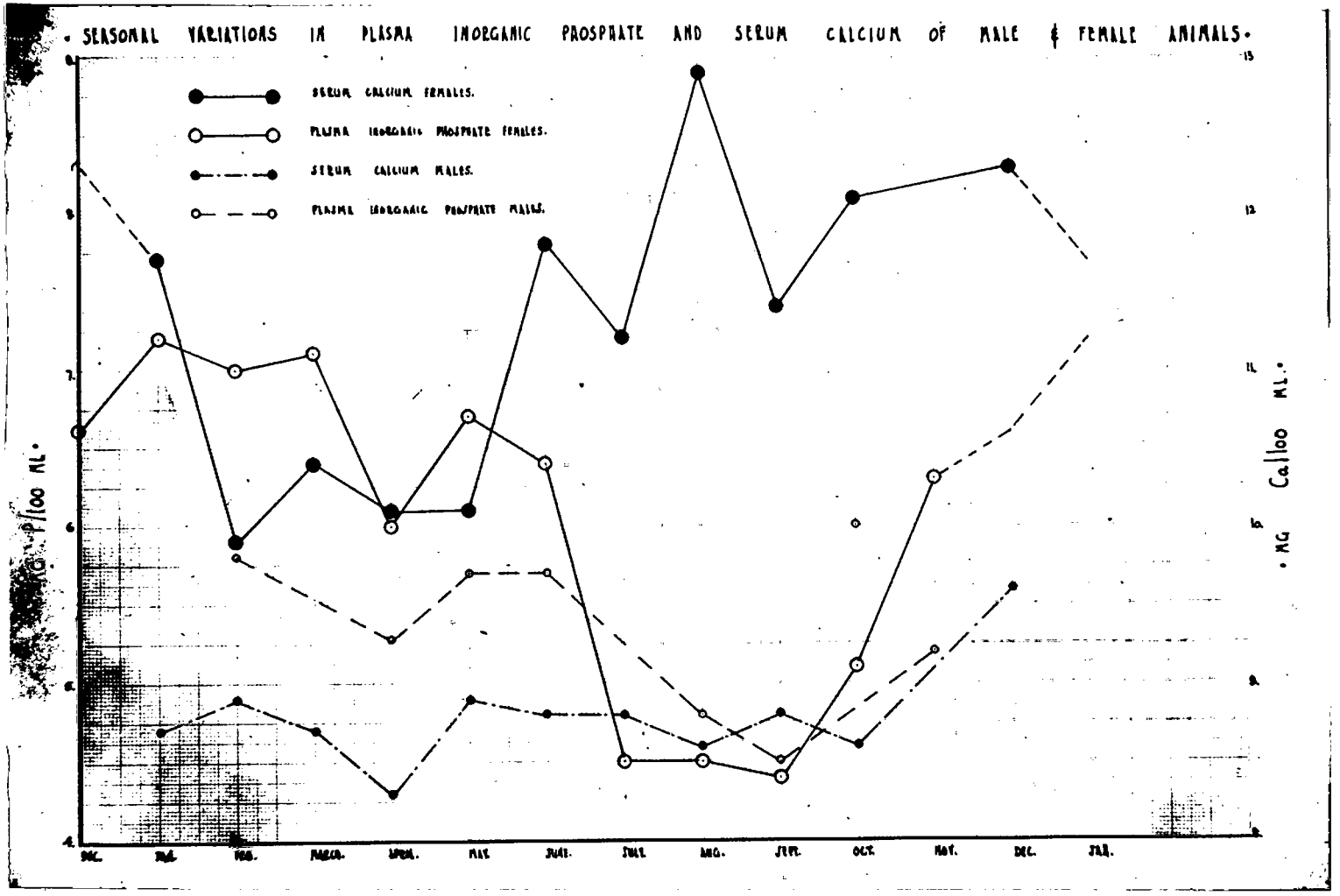
Plasma inorganic phosphate in males and females during the normal reproductive cycle.

Month	Females	Males
December, 1937	6.6 ± 0.24 (5)	
January, 1938	7.2 ± 0.15 (7)	
February	7.0 ± 0.10 (27)	5.9 ± 0.07 (20)
March	7.1 ± 0.17 (5)	
April	6.0 ± 0.07 (7)	5.4 ± 0.07 (7)
May	6.7 ± 0.17 (7)	5.8 ± 0.16 (5)
June	6.9 ± 0.09 (11)	5.7 ± 0.07 (7)
July	4.5 ± 0.06 (9)	4.4 ± 0.10 (9)
August	4.5 ± 0.11 (15)	4.8 ± 0.17 (6)
September	4.4 ± 0.20 (6)	4.5 ± 0.10 (7)
October	5.1 ± 0.13 (7)	
November	6.3 ± 0.26 (12)	5.2 ± 0.43 (5)

Each estimation required approximately 2 toads. Detailed figures appear in the appendix: protocol No. 4.

In Table VI the correlation between plasma inorganic phosphate and serum calcium is recorded.

Table VI. .....


SEASONAL VARIATIONS IN PLASMA INORGANIC PROSPHATE AND SERUM CALCIUM OF MALE & FEMALE ANIMALS.
SERUM CALCIUM FEMALES.
PLASMA INORGANIC PHOSPHATE FEMALES.
SERUM CALCIUM MALES.
PLASMA INORGANIC PHOSPHATE MALES.
MG P/100 ML.
MG Ca/100 ML.
DEC.
JAN.
FEB.
MARCH.
APRIL.
MAY.
JUNE.
JULY.
AUG.
SEPT.
OCT.
NOV.
DEC.
JAN.

GRAPH II.

TABLE VI.

Seasonal variations of plasma inorganic phosphate and serum calcium of male and female toads.

Month	Females		Males	
	(1938) Plasma inorg.P	(1933) Serum calcium	(1938) Plasma inorg.P	(1933) Serum Ca
January	7.2	11.7		8.7
February	7.0	9.9	5.9	
March	7.1	10.4		8.9
April	6.0	10.1	5.4	8.7
May	6.7	10.1	5.8	8.3
June	6.9	11.8	5.7	8.9
July	4.5	11.2	4.4	8.8
August	4.5	12.9	4.8	8.8
September	4.4	11.4	4.5	8.6
October	5.1	12.1		8.8
November	6.3		5.2	
December	6.6	12.3		9.6

The above data are graphically represented in Graph 11 below.

Discussion....

DISCUSSION.

The plasma inorganic phosphate levels of males and females.

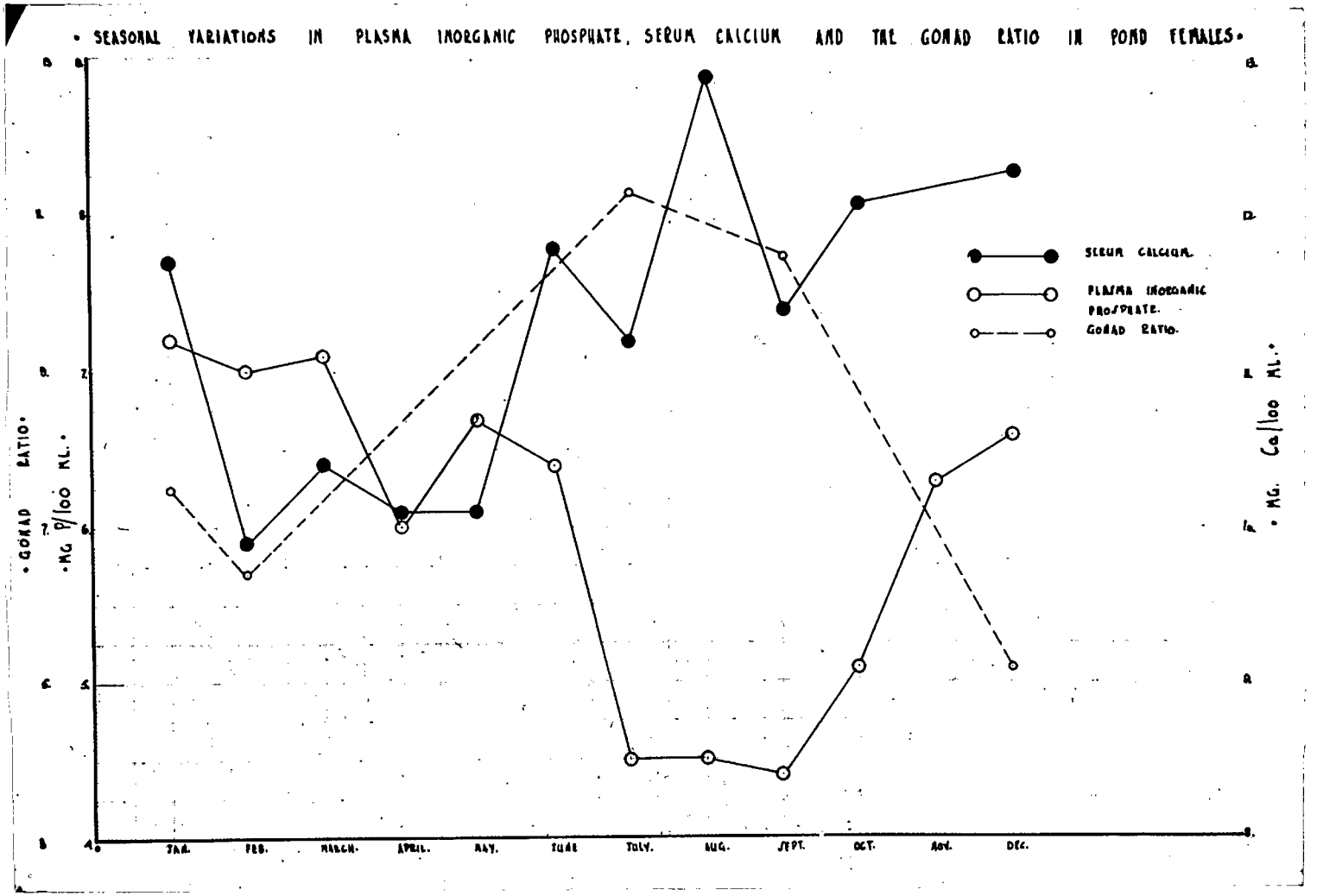
From Table V and Graph 11, it can be seen that a striking and significant difference in the plasma inorganic phosphate level of male and female toads exists, except during the breeding season (July - October). This is interesting in view of the fact that Charles (1931) and Shapiro (1933b) recorded a significant difference in serum calcium level of male and female toads. From Table VI and Graph 11 it will be seen that the difference in serum calcium between male and female toads is observed throughout the year and whereas the greatest sexual difference (46 per cent) is observed in August, soon after the beginning of the breeding season, there is no sexual difference in plasma inorganic phosphate at this time.

Seasonal changes in pond animals.

The plasma inorganic phosphate of female toads is highest in summer, autumn and early winter and lowest in late winter and early spring i.e. during the rainy and breeding seasons. The plasma inorganic phosphate returns to a high level throughout late spring and summer. It is very interesting to note how closely this follows changes in serum calcium. Serum calcium is highest in late winter and early spring and lowest in autumn and early winter (Graph 11). It will be seen that the reciprocal relation between calcium and inorganic phosphate is confirmed in these experiments

although .....

Although calcium and inorganic phosphate were both high in summer, too much emphasis must not be laid on this discrepancy. The results are all the more remarkable when it is remembered that the calcium determinations were made in 1932 and the inorganic phosphate in 1936 and that the atmospheric and temperature conditions naturally differed somewhat during these two years.


• SEASONAL VARIATIONS IN PLASMA INORGANIC PHOSPHATE, SERUM CALCIUM AND THE GONAD RATIO IN POND FEMALES.
• GONAD RATIO.
• MG P/100 ML.
• MG. Ca/100 ML.
SERUM CALCIUM.
PLASMA INORGANIC PHOSPHATE.
GONAD RATIO.
JAN.
FEB.
MARCH.
APRIL.
MAY.
JUNE
JULY.
AUG.
SEPT.
OCT.
NOV.
DEC.

Graph 111 shows the correlation between ovary/body weight serum calcium (Shapiro, 1933b) and plasma inorganic phosphate. (The ratio of the weight of the ovaries to the body weight of the animal from which they have been removed i.e. the ovary/body weight is termed the gonad ratio.) It is seen that up to the beginning of the breeding season, there is a striking correlation between the state of the ovaries (as judged by the gonad ratio) and the levels of serum calcium and plasma inorganic phosphate. There is a gradual increase in both ovary weight and serum calcium, the highest level for both being reached in July, the beginning of the breeding season. Concomitant with this there is a fall in plasmainorganic phosphate but this fall is more abrupt and is synchronous with the onset of breeding. After this the ovaries decline and lose weight as the ova are extruded in large numbers during the breeding season and this loss of weight continues during the summer months. The serum calcium is maintained at a high level during this period but the plasma inorganic phosphate remains low throughout the breeding season and when this is over it gradually rises to the normal anoestrous level. (Bork, 1936). Shapiro (1933b) concludes that the influences of the pituitary on ovarian and on calcium metabolism are concomitant but independent. It will be shown in the sequel that the seasonal changes in plasma inorganic phosphate are probably not due to ovarian activity and thus are more likely to be caused by

pituitary .....

pituitary activity.

The changes in the serum calcium and the plasma inorganic phosphate levels of Xenopus, except for time relations, are generally similar to those which occur in birds at the time of egg laying. Riddle and Reinhart (1926) found that the serum calcium of pigeons rose during the pre-ovulation phase to a value double that of the resting period. The high level was maintained during the greater part of the ovulation period and was succeeded by a post-ovulation fall to the normal resting level. These changes occupied in all about 10 days of the reproductive cycle. Sun and Macovan (1930) showed that the serum calcium of fowls rose considerably when egg laying commenced, a high average level bring maintained during the laying period to fall again when moulting commenced.

In Xenopus the eggs are not enclosed in a lime shell but Riddle and Reinhart (1926) found the increase in serum calcium in pigeons began several days before egg-shell formation began and came to the conclusion that it is in no wise necessarily correlated with the large calcium need for shell formation. They conclude that the very high calcium values accompanying each ovulation period is an expression of increased parathyroid activity. The rise and fall in serum calcium level in the fowl was found by Macovan (1932) to be associated with distinct histological changes in the parathyroids.

This is .....

This is in keeping with the results found with Xenopus. A secretion of parathyrotrophic principle just before the breeding season would lead to the stimulation of parathyroid secretion. This would produce a fall in inorganic phosphate (see sequel) and a rise in serum calcium (see introduction). When the breeding season is over the parathyroids are no longer stimulated and a rise in inorganic phosphate and fall in serum calcium results. These effects are in accord with the seasonal changes in plasma inorganic phosphate and serum calcium already described and this may be the responsible mechanism. That changes in the parathyroids, and this presumably in plasma inorganic phosphate and serum calcium, may be due to the result of differences in solar radiation will be discussed below (Higgins and Sheard, 1928). An important factor in determining the seasonal variation in plasma inorganic phosphate and serum calcium of the clawed toad in the natural surroundings may thus be the different amounts of sunlight at different times of the year. The changes in the parathyroid glands as a result of differences in solar radiation are probably effected through the intermediation of the pituitary gland. The seasonal changes in ovarian activity are probably also due to variations in the amount of light. Berk (1938) has recently shown the importance of climatic conditions particularly the rainfall and temperature in controlling the activity of the pituitary in Xenopus. He has

found .....

found that the rainfall and temperature cycles can be divided into three definite phases corresponding closely in time with the stages of the ovarian cycle and consequently with phases of anterior pituitary activity. It is probable that the pituitary not only produces gonadotrophic principles, but is stimulated to produce other factors such as the parathyrotrophic principle.

The seasonal changes in plasma inorganic phosphate and serum calcium raise a number of problems. These effects may be brought about by the pituitary, the gonads or the parathyroids. Other endocrine organs may also be involved. It is therefore necessary to determine the effects of the various endocrine glands on the plasma inorganic phosphate.

The plasma inorganic phosphate of males unlike that of the serum calcium exhibits changes similar to those occurring in female toads. These changes, however, are very much smaller. Shapiro (1933b) has found that the mineral metabolism of males is much more stable than that of females. Why there should be a discrepancy between serum calcium and plasma inorganic phosphate in this instance is not clear. It is probable that the pituitary of the male is stimulated by changes in light in its natural environment just as in the female, but that the changes in the blood are less well marked.

Changes in captivity.

From Tables lll and lV and Graph 1 it will be seen that the plasma inorganic phosphate of captive animals at first

falls ....

falls and remains at a low level for six months, after which it rises and remains at a high level for six months. It is very interesting to compare this with the effects of captivity on serum calcium. (Table III and Graph 1). Shapiro (1933b) states that at first serum calcium values for captive and pond animals follow each other but this is succeeded by a period during which the serum calcium values of captive animals rise. After this a steady and progressive decrease in the serum calcium of captive toads occurs and after 10 months this has reached a very low level. Thus although the experiments were performed at an interval of 4 years an exact reciprocal relation between calcium and inorganic phosphate can be seen.

Shapiro (1933b) found that the changes in serum calcium were accompanied through the period of captivity by a progressive involution of the ovaries and after 10 months of captivity the ovaries were gelatinous masses in which no individual ova could be seen, macroscopically. In the experiments on inorganic phosphate judging by the gonad ratio there was little change in the condition of the ovaries for as long as 6 months captivity although after this the gonad ratio tended to fall. It was found that the gonads kept in fairly good condition until about 13 months after captivity. This may be explained in view of the fact that Shapiro found his animals became emaciated and weak after 9 or 10 months captivity whereas in

the ....

the author's series of experiments this effect was not observed until 15 months of captivity.

A probable explanation of changes in the level of the serum calcium and the plasma inorganic phosphate during captivity is suggested by the results obtained by Higgins and Sheard (1928) on the effect of light on the parathyroid glands of chicks. Histological observations on the parathyroid glands of Xenopus unfortunately have not yet been made. Higgins and Sheard found that both the longer and shorter wave-lengths were essential for the maintenance of the normal parathyroid glands. In the absence of light of certain wave-lengths the parathyroid glands undergo hyperplastic changes within the first few weeks. The cells in the hyperplastic gland do not differ from those in the normal gland and they conclude that the cells are functioning and that secretory activity is accentuated so that the hormonal output is increased. At the conclusion of the third week regressive changes with the enlarged glands become evident. This involves destruction of the cords, shrinking of the cells and the development of cysts. They find further that the initial hyperplasia may be partially obviated by the addition of a small amount of cod-liver oil to the diet.

The results obtained in the experiments on serum calcium and plasma inorganic phosphate in Xenopus are partly susceptible of interpretation with reference to the phenomena

observed by Higgins and Shoard. The toads used for the investigation were kept in tanks in a dark corner of the laboratory and were thus exposed to dim light. Lack of sufficient solar radiation presumably had the same effects on the pituitary of the toad. First an initial hyperplasia of the parathyroid glands occurred leading, presumably, to increased hormonal output and a rise in serum calcium with a fall in plasma inorganic phosphate. Secondly, a progressive cystic degeneration of the glands which would account for the steady decrease in serum calcium and rise in plasma inorganic phosphate observed during the later months of captivity. (see Graph 1).

This is all the more interesting in view of the fact that it was noticed that animals brought into the laboratory during the breeding season when the plasma inorganic phosphate was already low, presumably due to parathyroid activity, did not show a preliminary fall in plasma inorganic phosphate. (e.g. see thyroidectomy controls, castration controls). Animals brought into the laboratory during the summer months had a high plasma inorganic phosphate level presumably due to parathyroid hypofunction and this level fell within a few weeks of captivity (see Table IV).

The absence of vitamin D from the diet probably was a contributory factor in the changes occurring in calcium and phosphorus metabolism during captivity. It is possible that

lack of vitamin D may have affected the serum calcium and plasma inorganic phosphate directly without hormonal intermediation since vitamin D is known to affect the intermediary metabolism of calcium and phosphorus (see introduction). A diet of raw minced meat, such as was fed to the toads is probably deficient not only in vitamin D but also in vitamins A and E. It is interesting to note that a marked degree of emaciation of female toads occurred after 10 months (Shapiro) or 15 months (present author) of captivity and it is possible that vitamin deficiency, particularly vitamin A deficiency played a part in this phenomenon.

The following results have recently been obtained showing the effect of light on pituitary activity. Koller and Rodewald (1934) demonstrated stimulation of skin colour changes in frogs, by means of light. This was produced through the hypophysis. Florentin and Stutinsky (1936) have found that the anterior pituitary of frogs kept in the dark seemed to lose their chromophobe cells and only a few basophils and many acidophils remained. Jores (1935) in a rabbit showed that periods of darkness raised the melanophore skin hormone in the blood and reduced it in the pituitary. Bissonette (1938) has reviewed this subject and states that modifications of sexual cycles in many amphibians, birds and mammals can be induced by altering the daily exposure to light, indicating that the anterior pituitary is affected

physiologically ...

physiologically by such changes. Studies on the pituitary glands of frogs, guinea pigs, mice, rats and rabbits show seasonal cyclic changes in cell types and endocrine activity of these glands associated with sexual changes.

Shapiro (1933b) commenting on the data of Hogben, Charles and Slome (1931) showed that light exerts an important influence on the captivity effect exhibited by the ovaries of the toad. No retrogressive changes occurred in the ovaries of normal toads even after 5 months captivity the animals being continually submitted to the rays of electric lights day and night. Quite a considerable retrogression had occurred in the ovaries of eyeless animals.

In the fieldmouse, Baker and Ranson (1932) have shown that reducing the daily hours of light from 15 to 9 hours almost prevents reproduction, the females mainly being affected.

The toads in captivity were subjected to temperature variations which were on the whole perhaps less extreme than variations in pond temperatures. While this may be an additional factor affecting pituitary and ovarian activity (Berk, 1938), the present author has shown that a difference of 15°C has no effect on the level of the plasma inorganic phosphate and Shapiro (1933b) has shown that a difference of 20°C has no effect on the serum calcium level.

Summary .....

SUMMARY.

1. Experiments are described to show that a range of 15°C does not affect the level of plasma inorganic phosphate of the South African clawed toad (Xenopus laevis).

2. Whereas the serum calcium level is higher in females than in males throughout the normal reproductive cycle, the plasma inorganic phosphate is higher in females only during the pro-oestrous and anoestrous periods as defined by Berk (1936).

3. The reproductive cycle in females is associated with changes in serum calcium and plasma inorganic phosphate levels and in ovarian activity. In male toads the plasma inorganic phosphate falls slightly during the breeding season just as in female toads but as this fall is not marked the plasma inorganic phosphate of male and female toads are the same during the breeding season. The serum calcium of male toads, in contrast, remains remarkably steady.

4. Captivity first causes a fall, then a steadily maintained elevated plasma inorganic phosphate. Serum calcium first shows a rise then steadily declines in female toads.

5. It is suggested that seasonal variations in the amount of solar radiations and inadequate illumination during captivity may be important agencies influencing the plasma inorganic phosphate and the serum calcium through the parathyroid glands and the pituitary; and perhaps ovarian activity through the anterior .....

anterior lobe of the hypophysis.

6. Changes occurring due to seasonal variations and captivity necessitate investigation of the effect of the endocrine glands on plasma inorganic phosphate, particularly the pituitary, parathyroids and the gonads.

THE EFFECT OF THE PITUITARY ON THE PLASMA INORGANIC PHOSPHATE.

C. THE PITUITARY.

(a) Hypophysectomy.

Animals were hypophysectomised as described according to the method of Hogben (1923). They were kept in the laboratory and killed at monthly intervals. After 7 months it was found that the colour response reappeared and these animals were considered to have regenerated the pars tuberalis (Hogben and Slome, 1931).

The plasma inorganic phosphate was determined on three types of hypophysectomised animals.

(1) Both lobes removed.

(2) Anterior lobe (including pars tuberalis) removed.

(3) Regenerated pars tuberalis but pars anterior absent.

Operative controls, prepared as previously described, were used for the first 4 months to control the hypophysectomised animals. After this period animals kept in captivity and from the same original batch as the hypophysectomised animals were used as controls. These captive animals were kept under exactly the same conditions throughout the experiment as the hypophysectomised animals. In all the investigation proceeded for a period of 9 months.

The results of hypophysectomy are recorded below in Table VII.

Table VII ....

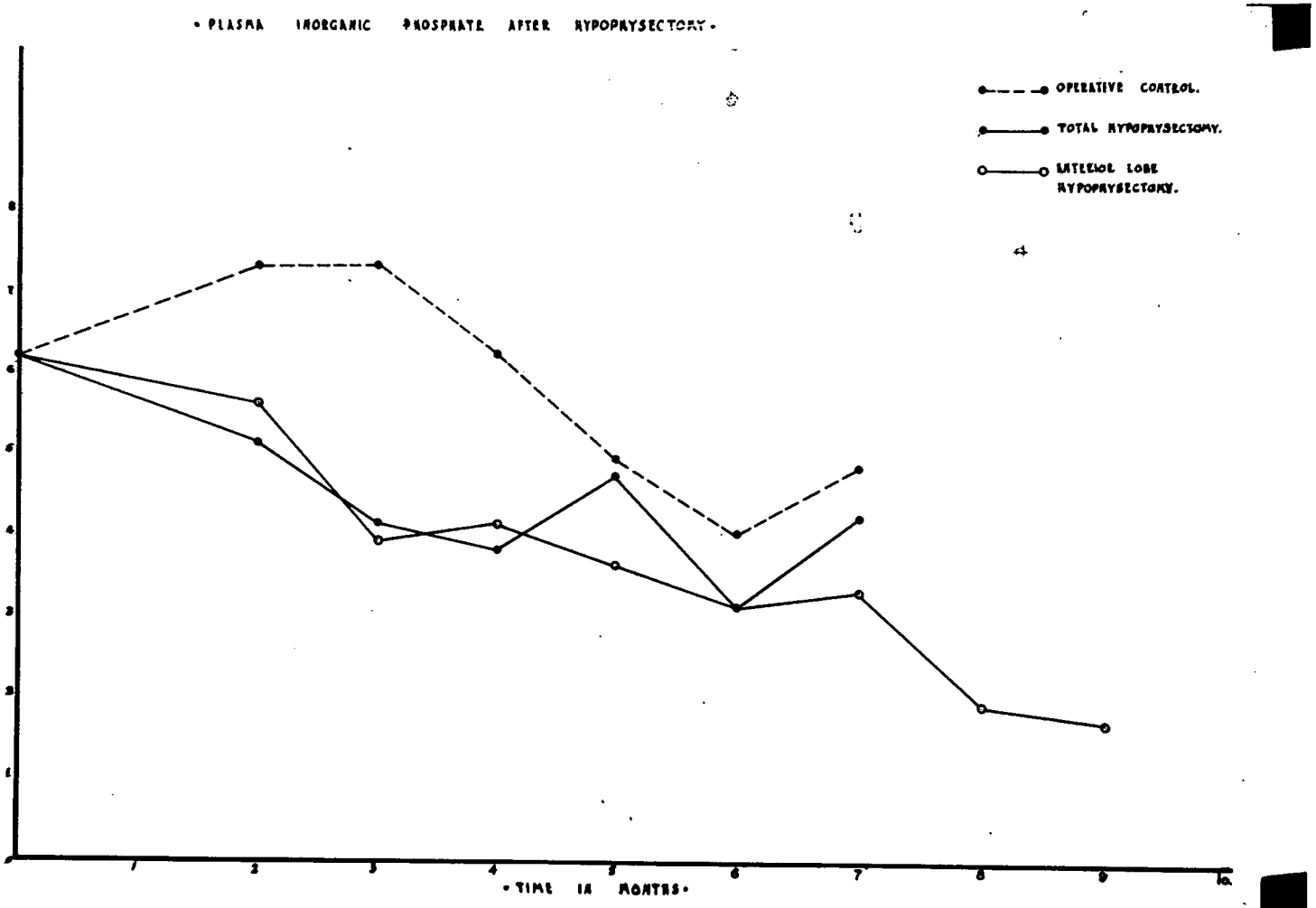

• PLASMA INORGANIC PHOSPHATE AFTER HYPOPHYSECTOMY •
OPERATIVE CONTROL.
TOTAL HYPOPHYSECTOMY.
ANTERIOR LOBE HYPOPHYSECTOMY.
• TIME IN MONTHS •

TABLE VII.

The effect of hypophysectomy and regeneration on the plasma inorganic phosphate in female toads.

Preoperative Normal 6.2 ± 0.21 (5)

Months after removal	Anterior Lobe removed	Both lobes removed	Operative Controls	Regenerated pars tubera
2	5.6±1.13 (3)	5.1±0.20(2)	7.3 ± 0.34 (3)	
3	3.9±0.44 (3)	4.1±0.51(3)	7.3 ± 0.20 (2)	
4	4.1±0.22 (3)	3.8±0.17(4)	6.2 ± 0.19 (3)	
5	3.6±0.17 (4)	4.7±0.21(4)	4.9 ± 0.09 (4)	
6	3.1±0.25 (5)	3.1±0.24(4)	4.0 ± 0.23 (4)	
7	3.3±0.27 (6)	4.2±0.35(3)	4.8 ± 0.20 (2)	
8				1.9±0 (4)
9				1.7±0.09(5)

Captivity controls

About 14 dozen toads were used for the experiment. Each estimation required about 2 animals. Detailed figures appear in the appendix: protocol No. 5.

These results show the effect of hypophysectomy on the plasma inorganic phosphate but the earliest experiment was done two months after hypophysectomy. In Table VIII more acute effects are shown shortly after operation.

Table VIII ....

TABLE VIII.

Plasma inorganic phosphate shortly after hypophysectomy.

Days after operation	Anterior lobe removed	Both lobes removed	Operative Controls
3	4.4±0.12 (3)		5.3 ± 0.25 (2)
4	5.0±0.17 (5)		
7	5.1±0.18 (5)	4.5±0.37 (7)	5.7±0.35 (7)
8			5.9±0.57 (3)
14	4.6±0.04 (6)	4.5±0.33 (4)	5.6±0.13 (4)
21	3.5±0.10 (5)	3.4±0.06 (5)	5.9±0.18 (6)

Captivity controls

About 12 dozen toads were used. Each estimation represents about 2 animals. Detailed figures appear in the appendix: protocol No. 16.

(b) Injection of sheep anterior pituitary extract into anterior lobe hypophysectomised females.

Anterior pituitary and brain extracts were prepared as already described. This extract is rich in gonadotrophic activity.

The extracts were then estimated for their inorganic phosphate content and it was found that the anterior pituitary extract contained slightly more than the brain extract. The inorganic phosphate value of the anterior pituitary extract was approx-

imately ....

imately 29.0 mgs/100 ml. At first the proteins were precipitated with alcohol to eliminate some of the phosphate and it was found that the inorganic phosphate content was now 5 mgs/100 ml. This extract was injected into animals and it was found that ovulation no longer occurred i.e. the gonadotrophic substance had been removed by the alcohol. Alcohol precipitation was therefore discarded.

The proposed amount of anterior pituitary extract to be injected was equivalent to one half a sheep's anterior pituitary and it was found that this was contained in 0.5 ml. fluid. This amount of extract contained 0.15 mg. inorganic phosphate. The average toad has about 3 ml blood and an average inorganic phosphate value is 6 mgs P/100 ml (plasma and whole blood contain about the same amount of inorganic phophate, see introduction). The average amount of inorganic phosphate in a toad's blood is thus about 0.18 mgs and this amount was being injected into the animal.

The experiment, therefore, was controlled as follows. A sample of potassium phosphate solution (diluted from the stock phosphate solution) was prepared so that 0.5 ml contained 0.14 mg P. A batch of toads was divided into two sections half of which received 0.5 ml phosphate solution containing 0.14 mgs P per animal, the other half receiving 0.5 ml distilled water per animal. All the injections were given intraperitoneally.

Table IX shows the effect of phosphate injection on the plasma inorganic phosphate.

Table IX .....

TABLE 1X.

The effect of Potassium phosphate (0.14 mg.), injected intraperitoneally on the plasma inorganic phosphate.

Time in hours after injection	Potassium phosphate	Controls Distilled water
24	5.3 (4)	5.7 (4)
48	5.4 (6)	5.1 (7)

Pre-injection level 6.8 (6)

Time in hours after injection	Potassium phosphate	Controls Distilled water
6	6.3 (7)	5.8 (5)
15	6.6 (3)	7.1 (6)

7 Dozen pond toads were used. Each estimation required approximately 1.5 animals. The detailed figures appear in appendix;protocol No. 7.

From the above table it can be seen that inorganic phosphate injected intraperitoneally in the quantities present in anterior pituitary extract had no effect on the plasma inorganic phosphate. Estimations were made at varying times after injection, the times chosen being the same as those

after .....

after anterior pituitary injection into normal, hypophysectomised and castrated animals. It will be observed that after 6 hours the toads injected with distilled water showed a plasma inorganic phosphate value of 5.6. This represents a fall from 6.8. Animals injected with phosphate solution showed a fall to 6.3. This effect is probably due to dilution of the plasma by the fluid injected which is quickly absorbed and as quickly eliminated. This same phenomenon was observed with anterior pituitary extract and insulin injections and will be discussed in the relevant sections That intraperitoneal injection of phosphate in amounts equal to the total inorganic phosphate content of the blood produced no change in plasma inorganic phosphate need occasion no surprise. The amount of inorganic phosphate in the blood is only a minute fraction of the phosphate content of the body. Large reservoirs of inorganic phosphate are present such as the bones, soft tissues and organic phosphate compounds and any influx of phosphate can rapidly be dealt with. Bolliger and Hartman (1925) found that injection of phosphate into normal and depancreatised dogs produced an initial rise in blood inorganic phosphate with a return to normal. Lehman (1921) found an increase of 4 to 5 times the concentration of inorganic phosphate in rabbit's blood with a return to normal in 4 hours.

In these experiments large quantities about 175 mg. per kilo

and ....

and 75 mg. per kilo respectively were injected.

Since intraperitoneal injection of inorganic phosphate had no effect on the plasma inorganic phosphate, brain extract only was used to control injections of anterior pituitary extract.

Animals were hypophysectomised according to the method of Hogben (1923) as already described. The anterior lobe only was removed. The storage and feeding of animals has already been described.

After 2 months the animals were used for injection experiments. Two months was chosen as a fairly marked hypophysectomy effect is observed by that time and no regeneration of the pars tuberalis was observed in this time.

Extracts of anterior pituitary and of brain were made as before and 0.5 ml extract injected intraperitoneally in each toad.

In all 10 dozen anterior lobe hypophysectomised animals were injected with anterior pituitary extract and the same number with brain extract.

In Table X the effect of S.A.P.2A on anterior lobe hypophysectomised animals is shown.

TABLE X. .....

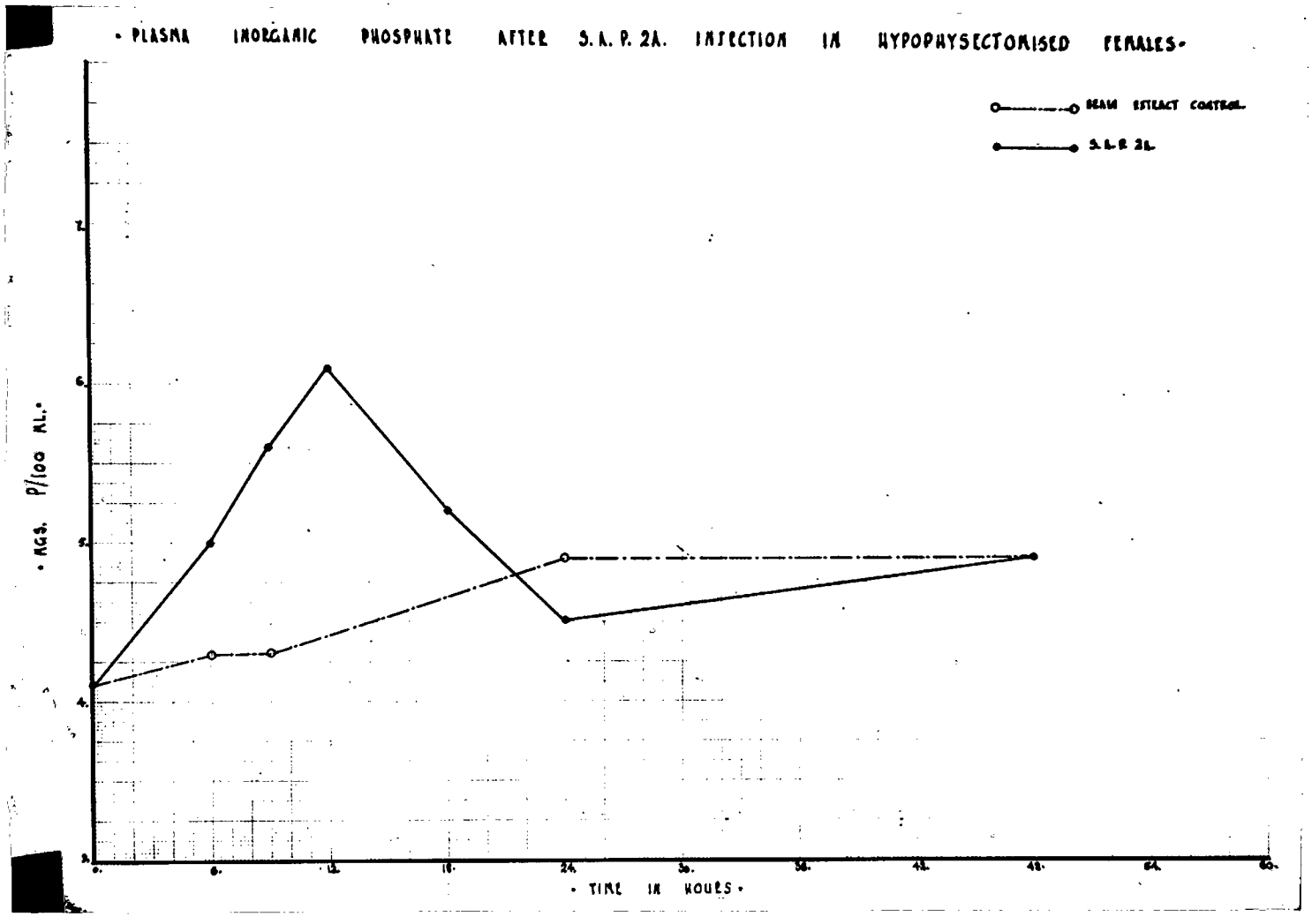

• PLASMA INORGANIC PHOSPHATE AFTER S. A. P. 2A. INJECTION IN HYPOPHYSECTOMISED FEMALES.
BEAM EXTRACT CONTROL.
S.A.P. 2A.
• MGS. P/100 ML.
• TIME IN HOURS •
0.
6.
12.
18.
24.
30.
36.
42.
48.
54.
60.

CHART V.

TABLE X.

Plasma inorganic phosphate after injection of S.A.P.2A into anterior lobe hypophysectomised animals.

Pre-operative normal 6.6 ± 0.24 (5)

Hypophysectomised animals 4.1 ± 0.07 (4)

Time in hours after injection	S.A.P.2A	Brain Extract
6	5.0 ± 0.12 (6)	4.3 ± 0.05 (5)
9	5.6 ± 0.19 (6)	4.3 ± 0.13 (6)
12	6.1 ± 0.21 (4)	※
18	5.2 ± 0.31 (3)	※
24	4.5 ± 0.10 (5)	4.9 ± 0.13 (4)
46	4.9 ± 0.47 (4)	4.9 ± 0.22 (4)

※ Animals escaped.

5 Dozen animals were used. On the average each estimation required 1.5 toads. The detailed figures appear in the appendix:protocol No. 8.

The above results are graphically represented below (Graph V).

In this particular experiment the subsequent fall in plasma inorganic phosphate was not observed. The experiment was therefore repeated.

Table XI shows the effect of S.A.P.3A on anterior lobe hypophysectomised animals.

TABLE XI .....

TABLE XI.

Plasma inorganic phosphate after injection of S.A.P.3A into anterior lobe hypophysectomised animals.

Pre-injection level 5.4 ± 0.25 (3)

Time in hours after injection	S.A.P.3A	Brain Extract
12	6.0 ± 0.14 (4)	4.7 ± 0.07 (4)
24	5.7 ± 0.23 (3)	4.3 ± 0.14 (4)
36	3.0 ± 0.15 (4)	3.6 ± 0.15 (4)
48	3.2 ± 0.19 (5)	5.1 ± 0.35 (3)

4.5 dozen animals were used for this experiment. On the average each estimation required 2 hours. The detailed figures appear in the appendix:protocol No. 9.

TABLE XII.

Plasma inorganic phosphate after injection of S.A.P.4A into anterior lobe hypophysectomised animals.

Pre-injection level 4.8 ± 0.28 (4)

Time in hours after injection	S.A.P.4A	Brain Extract
6	6.2 ± 0.67 (4)	4.0 ± 0.06 (4)
12	4.7 ± 0.15 (3)	4.3 ± 0.00 (2)
36	3.8 ± 0.15 (4)	4.7 ± 0.44 (3)

5 dozen .....

5 dozen toads were used. On the average each estimation required 3 animals. The detailed figures appear in the appendix:protocol No. 9.

Ovulation took place in most of the animals injected with S.A.P.2A, S.A.P.3A., and S.A.P.4A., but ovulation and oviposition had no relation to changes in plasma inorganic phosphate. Oviposition was not as profuse as in fresh animals.

(c) Injection of sheep anterior pituitary extract into pond females.

Fresh pond females were kept in the laboratory for one week before injection.

Each of 71 animals was injected intraperitoneally with 0.5 ml S.A.P.1A. and 0.5 ml brain extract was injected as a control into each of 73 toads. The results are recorded in Table XIII below.

TABLE XIII. ....

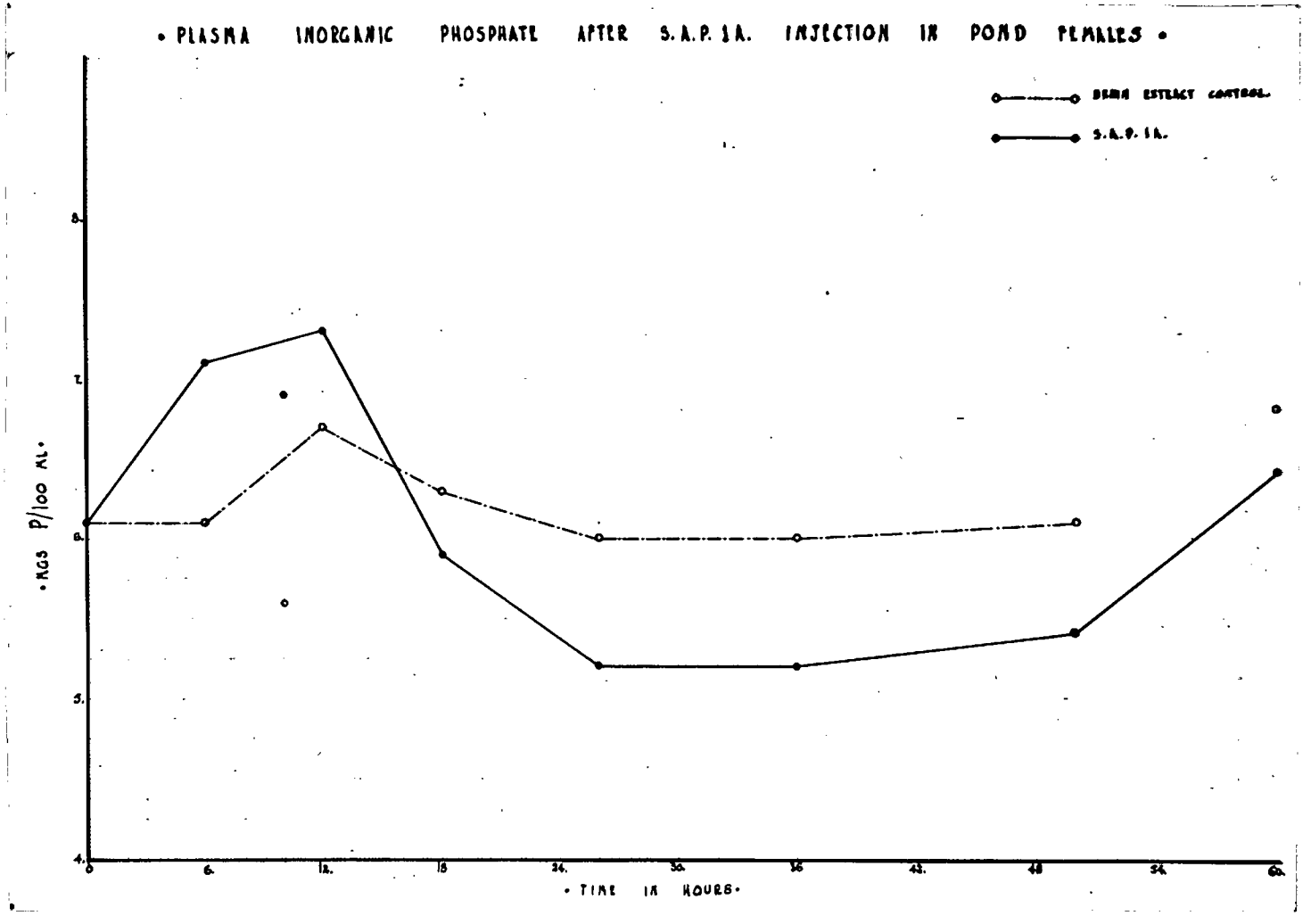

• PLASMA INORGANIC PHOSPHATE AFTER S.A.P.1A. INJECTION IN POND FEMALES •
BEMA ESTRACT CONTROL.
S.A.P. 1A.
• MGS P/100 ML •
8.
7.
6.
5.
4.
0
6.
12.
18
24.
30.
36
42.
48
54.
60.
• TIME IN HOURS •

TABLE XIII.

Plasma inorganic phosphate after injection of S.A.P.1A. into normal animals.

Pre-injection level 6.1 ± 0.10 (6)

Time in hours after injection	S.A.P.1A.	Brain Extract
6	7.1 ± 0.08 (6)	6.1 ± 0.12 (6)
10	6.9 ± 0.09 (6)	5.6 ± 0.17 (5)
12	7.3 ± 0.17 (6)	6.7 ± 0.16 (7)
18	5.9 ± 0.18 (7)	6.3 ± 0.09 (6)
26	5.2 ± 0.14 (7)	6.0 ± 0.12 (7)
36	5.2 ± 0.13 (7)	6.0 ± 0.14 (6)
50	5.4 ± 0.12 (9)	6.1 ± 0.12 (6)
60	6.4 ± 0.09 (5)	6.6 ± 0.06 (4)

13 Dozen toads were used. Each estimation represents approximately 1.5 animals. The detailed figures appear in the appendix:protocol No. 10.

The above results are graphically represented below (Graph VI).

Most of the animals injected with anterior pituitary extract oviposited profusely within 24 hours. Changes in plasma inorganic phosphate, however, occurred before ovulation took place. (Very few animals ovulated as early as 6 hours after injection. See gonads).

Discussion .....

DISCUSSION.

Hypophysectomy.

From an inspection of Table VII and Graph IV, it is seen that the controls showed the captivity effect already described. From the fifth month onwards, when captive controls were used a marked fall in plasma inorganic phosphate was noticed. If the experiment had continued a subsequent rise could have been anticipated. It will be observed that during the first 4 months when operative controls were used the plasma inorganic phosphate remained at about the normal level.

Hypophysectomy produced a pronounced effect on the plasma inorganic phosphate. In the first few months there was a fall of 34 per cent as compared with the controls. This effect of hypophysectomy though observed throughout the experiment was not as well marked towards the end of the experiment when the captivity effect became manifest.

Since removal of both lobes of the pituitary had no greater effect than removal of the anterior lobe alone it is probable that the effect of hypophysectomy was due to anterior lobe deficiency alone. It is interesting to compare the effect of the pituitary on calcium and inorganic phosphate of Xenopus. Shapiro (1933b) has shown that anterior lobe hypophysectomy produced a fall in serum calcium whereas removal of both lobes produced a less intense fall and injection of posterior pituitary extract produces

a rise .....

a rise. The plasma inorganic phosphate therefore does not show analogous changes. Removal of the anterior lobe produces a fall in both calcium and plasma inorganic phosphate in Xenopus, i.e. there is no reciprocal rise and fall of calcium and inorganic phosphate as is seen with other endocrine organs such as the parathyroid glands. Whereas the posterior lobe had no effect on plasma inorganic phosphate, it affected the level of serum calcium.

The effects of regeneration also differ with the two substances. Regeneration produced a still further fall in plasma inorganic phosphate. Figures as low as 1.9 and 1.7 mg./100 ml, have only been encountered as a result of hypophysectomy with regeneration.

Shapiro (1933b) has shown that the regenerated pars tuberalis of the anterior lobe caused a rise in serum calcium and the fall in hypophysectomy was due to removal of the pars tuberalis. An analogous effect on plasma inorganic phosphate was not observed. Too much emphasis, however, must not be laid on these figures showing the effect of regenerated pars tuberalis on plasma inorganic phosphate as they represent about 16 animals only. This particular experiment has not been repeated and the author feels that the results require confirmation before attempting an interpretation.

Table VIII shows the effects of hypophysectomy shortly after operation for periods varying from 3 - 21 days. Again it will be observed that there is no significant difference

between ...

between the plasma inorganic phosphate values of anterior lobe and total hypophysectomy so that the effects of hypophysectomy can be ascribed to the anterior pituitary alone. From this table it will be seen that operative controls do not show much variation, averaging 5.7 0.14 for 22 estimations. The anterior lobe hypophysectomised toads showed the following fall:-

(5.7 i.e. average control) - 4.4 - 5.1 - 4.6 - 3.5 and totally hypophysectomised toads a fall to:-

5.0 - 4.5 - 4.3 - 3.4.

It seems probable from these figures that there is a tendency for the plasma inorganic phosphate to fall within the first week of hypophysectomy but this fall only becomes significant 2 to 3 weeks after hypophysectomy. The significant difference between hypophysectomised and operative controls calculated for 7, 14 and 21 days respectively after operation are 20:1, 100:1, 100:1. The formula used to obtain the significant difference is $\frac{m_1 - m_2}{e^2_1 \; e^2_2}$ where m_1 and m_2 are the two mean results, and e_1 and e_2 are their respective standard errors (Burn, 1937).

Anterior pituitary extract into hypophysectomised animals.

Examination of Table X and Graph V shows that injection of anterior pituitary extract into anterior lobe hypophysectomised animals produced a rise in plasma inorganic

phosphate .....

phosphate almost to the preoperative level (33 per cent). This was followed by a fall to the hypophysectomised level. In this particular experiment the subsequent fall below the preinjection level was not observed.

In Table XI data of a repeat experiment show that after 36 and 48 hours the plasma inorganic phosphate of animals injected with anterior pituitary extract is significantly lower than the controls. These results are open to criticism in view of the unaccountable fall observed in the controls, after 36 hours. Table XII, however, shows a fall in plasma inorganic phosphate in the anterior pituitary extract injected but not in the controls after 36 hours.
Tables XI and XII again show the diluting effect of the extract on the blood plasma. Within 48 and 36 hours respectively the normal level had again been reached.

Anterior pituitary extract in normal animals.

Examination of Table XIII and Graph VI reveals the effect of sheep anterior pituitary extract on the plasma inorganic phosphate of normal animals fresh from the pond. It is seen that there is a rise of plasma inorganic phosphate of 10 - 23 per cent from 6 - 12 hours after injection, followed by a fall of 6 - 13 per cent from 18 - 50 hours after injection, then a return to normal in 60 hours.

The controls injected with brain extract showed little variation averaging 6.2 0.06 for 49 estimations. In Table XIV below is shown the change in plasma inorganic phosphate in animals injected with anterior pituitary extract and the significance of this change calculated from the formula.

TABLE XIV.

The significance of changes in plasma inorganic phosphate following S.A.P.1A.

Time in hours after injection	6	10	12	18	26	36	50	60
S.A.P.1A.	7.1	6.9	7.3	5.9	5.2	5.2	5.4	6.4
Significant difference	>100:1	>100:1	50:1	15:1	>100:1	>100:1	>100:1	

If Table XIII is examined it will be seen that after 10 hours the control plasma inorganic phosphate value was 5.6. This fall is similar to that occurring after 6 hours in Table IX. This fall may likewise be attributed to dilution of the plasma by the fluid injected. This phenomenon has been referred to before.

Conclusions.

From the series of experiments described the following conclusions can be drawn.

(1) Hypophysectomy leads to a fall in plasma inorganic phosphate and this may be observed within one week after operation and becomes fairly well marked in two to three weeks.

(2) Anterior pituitary extract produces an initial rise in plasma inorganic phosphate both in normal and in anterior

lobe .....

lobe hypophysectomised animals. This is succeeded by a fall below the pre-injection level.

Mode of action.

The results obtained after hypophysectomy show that the progressive fall in plasma inorganic phosphate is due to the removal of the anterior pituitary alone. Consequently in this discussion only the anterior pituitary is considered. Shapiro and Zwarenstein (1933) have shown, on the other hand, that removal of the anterior lobe of the pituitary leads to a significantly lower serum calcium level than removal of the entire gland. This does not occur with plasma inorganic phosphate. Injection of "antuitrin" (Parke, Davis and Co.) into completely hypophysectomised animals causes a rise, injection of pituitrin a fall in the level of serum calcium. Since both serum calcium and plasma inorganic phosphate fall after hypophysectomy and both rise after anterior pituitary injection it is likely that these effects are produced in different ways. As a rule substances affecting calcium and phosphorus affect them so that they vary inversely proportional to each other (see introduction).

In the introduction the possible modes of action of the anterior pituitary have been discussed viz. absorption, intermediary metabolism and excretion.
Some of the experiments on anterior pituitary injection were carried out in animals during the post-absorptive state and it is thus unlikely that plasma inorganic phosphate is

affected .....

affected by absorption of phosphate. (see introduction).
The possible modes of action may thus be summarised:-

(1) An effect on the plasma constituents (direct or indirect)

(2) An indirect effect following changes in carbohydrate metabolism

(3) An indirect effect following stimulation of some other endocrine gland.

(a) The Adrenal cortex

(b) The Gonads

(c) The Thyroid

(d) The Parathyroids

(4) An effect on the renal threshold for phosphate.

Brull and Eichholtz (1925), Brull (1937) have shown that hypophysectomy leads to a raised renal threshold for phosphate and consequently a decreased urinary phosphate output and raised blood inorganic phosphate value. These findings refer to events within a few hours after operation in dogs. In toads hypophysectomy leads to a depressed plasma inorganic phosphate and this lasts for as long as 9 months. In the toad, therefore, hypophysectomy cannot raise the renal threshold for phosphate. It is possible, on the other hand that the renal threshold for phosphate is lowered by hypophysectomy. No urine studies were made and thus this possibility cannot be entirely disregarded. If this hypothesis is true then injection of anterior pituitary extract raises the renal threshold. At the same time due to other

effects of the crude preparation injected secondary stimulation of some other gland might occur e.g. the parathyroid. Secretion from this gland would then lower the renal threshold (see introduction).

The fall in plasma inorganic phosphate is not due to degeneration of the ovaries. Two to three weeks after hypophysectomy the ovaries are in good condition in fact after 2 to 3 months little change was observed, yet the plasma inorganic phosphate decreased within two weeks after hypophysectomy.

To eliminate the influence of the ovary, however, experiments showing the effects of ovariectomy and of anterior pituitary injection into ovariectomised animals must be done. These will be described in the sequel. The results obtained show that the effect of hypophysectomy on plasma inorganic phosphate cannot be explained by ovarian hypofunction. Similarly effects of anterior pituitary injection (although the extracts were rich in gonadotrophic activity) did not produce changes in plasma inorganic phosphate by stimulating the ovaries.

It is unlikely that the thyroid gland atrophies sufficiently in 14 days to account for the marked fall in plasma inorganic phosphate. To eliminate the thyroid, however, experiments are required to show the effect of thyroidectomy and thyroid injection. These will be described in the sequel.

It was ....

It was found (see under thyroid gland) that thyroidectomy produced no change in plasma inorganic phosphate so that the fall in plasma inorganic phosphate occurring after hypophysectomy cannot be due to thyroid hypofunction.

Parathyroidectomy leads to a rise in inorganic phosphate and a fall in serum calcium (see introduction). The fall in serum calcium (Shapiro and Zwarenstein, 1933) following hypophysectomy may be due to parathyroid deficiency, but the change in plasma inorganic phosphate cannot be attributed to this. The rapidity of the onset of the fall in plasma inorganic phosphate following hypophysectomy and the immediate rise after injection of anterior pituitary extract is also against involvement of the parathyroid glands.

The following possibilities are therefore left:-

(1) An action on the blood constituents

(2) An indirect action on carbohydrate metabolism either through the anterior pituitary alone or together with the adrenal cortex.

The fact that removal of the anterior pituitary is reflected rapidly in the plasma inorganic phosphate level and that injections produce an immediate rise in inorganic phosphate (within 6 hours) in a cold blooded animal, suggests that the action is a direct one on the plasma constituents rather than an indirect one through changes in carbohydrate metabolism (the literature has already been discussed, see introduction).

Hypophysectomy leads to very little actual disturbance in carbohydrate metabolism if the animal is well fed (see introduction). One of the complex factors regulating carbohydrate metabolism is, however, upset. The anterior pituitary is one of the main factors antagonizing the action of insulin. Young (1938b) has prepared extracts of anterior pituitary containing glycotrophic or anti-insulin factor and states that the action of anterior pituitary extract is to inhibit the peripheral action of insulin. Himsworth and Scott (1938) found that the liver was not directly concerned in the antagonizing of insulin. de Wesselow and Griffiths (1936) have shown that the blood of "insulin resistant" diabetic patients possesses glycotrophic activity. It is possible that in hypophysectomised animals insulin is acting unopposed and is keeping the blood inorganic phosphate depressed. This should be reflected in the blood sugar level. The blood sugar level is more or less normal, unless the animal is starving. In the author's experiments the animals were well fed. Unfortunately no blood sugar estimations on hypophysectomised animals were done. The literature on this subject is enormous and most investigators agree that the blood sugar level of well-fed hypophysectomised animals is normal. Zwarenstein and Bosman (1932), however, have shown that in Xenopus laevis hypophysectomy whether total or anterior lobe only, had no effect on the fasting blood sugar. Hypophysectomy led to

a .....

a considerable increase in the tolerance to sugar.

The evidence presented shows that the anterior pituitary plays an important part in the regulation of the normal blood inorganic phosphate level. Hypophysectomy leads to a fall in plasma inorganic phosphate and injection of anterior pituitary extract a rise in plasma inorganic phosphate. Whether it does this through a factor directly affecting the mineral constituents of the blood, or through its effects on carbohydrate metabolism is difficult to determine. It appears more likely, however, that the effect is on the mineral constituents of the blood. This is further discussed in the sequel.

The subsequent fall of plasma inorganic phosphate following injection of anterior pituitary extract may be produced by several factors. It appears to be of a secondary nature and is probably produced by stimulation of some other endocrine gland. The extract injected was comparatively crude and probably contained a number of factors including gonadotrophic substance. The parathyroid glands may possibly be stimulated.

Gerschman and Marenzi (1935a) found a rise in blood inorganic phosphate following injections of anterior pituitary extracts into normal and thyroidectomised animals. If injected into thyro-parathyroidectomised animals there was very little change in inorganic phosphate. They attribute the effects of anterior pituitary extracts on blood inorganic

phosphate .....

phosphate to stimulation of the parathyroids. In Xenopus the effect of parathyroid is to produce a fall in blood inorganic phosphate (see under parathyroid glands). The secondary fall in plasma inorganic phosphate may be due to stimulation of the parathyroid glands. Similarly it may be due to stimulation of the thyroid gland (see under thyroid gland).

It is well known that injections of anterior pituitary produce a hyperglycaemia and may even lead to a persistent diabetes (Young, 1936a). The insulin reflexly secreted may reflexly produce a fall in blood sugar and also a fall in inorganic phosphate. Gorschman and Marenzi (1935b) showed that injection of anterior pituitary extract produced a rise of blood inorganic phosphate in normal animals but the rise is very much more marked in pancreatectomised animals. This they attributed to hypoparathyroidism but it is probable that the effect is due to the absence of a reflex secretion of insulin which would tend to keep the inorganic phosphate level depressed.

A possible explanation of the action of the anterior pituitary on plasma inorganic phosphate is that it produces a rise by virtue of an action on the mineral constituents of the plasma. As it also produces a hyperglycaemia, a reflex secretion of insulin occurs. When the

insulin secreted is sufficient (in some cases it is not) it overcomes the action of the extract and produces a fall in inorganic phosphate. To confirm this it is necessary to perform experiments on the effect of anterior pituitary extract on pancreatectomised animals (see sequel).

SUMMARY.

(1) Removal of the anterior lobe (i.e. pars anterior and pars tuberalis) of the pituitary leads to a significantly lower plasma inorganic phosphate level than in operative controls.

(2) When the pars tuberalis regenerates the plasma inorganic phosphate remains low in spite of the return of serum calcium to the normal level. The pars tuberalis is therefore not involved as it is with calcium.

(3) The progressive fall in plasma inorganic phosphate is due to the removal of the anterior lobe of the pituitary alone.

(4) The fall in plasma inorganic phosphate may be observed within one week of hypophysectomy and is fairly well marked within 2 to 3 weeks.

(5) Injection of anterior pituitary extract into anterior lobe hypophysectomised animals produces a rise of plasma inorganic phosphate to the preoperative level then a fall below the pre-injection level.

(6) Injection of anterior pituitary extract into normal animals produces an initial rise of plasma inorganic phosphate followed by a fall below the pre-injection level.

(7) The anterior pituitary affects calcium and inorganic phosphate in the same direction and it is probable that these two substances are affected through different mechanisms. The fall in serum calcium following hypophysectomy may .....

may be due to hypoparathyroidism but this cannot explain the fall in plasma inorganic phosphate.

(8) The possible modes of action of the anterior lobe of the pituitary are:-

(a) By raising the renal threshold to phosphate and thus producing a fall of plasma inorganic phosphate on hypophysectomy and opposite effects with anterior pituitary extract injections.

(b) By altering the plasma constituents.

(c) By affecting carbohydrate and consequently phosphorus metabolism.

(9) The parathyroids, the thyroid and the gonads are probably not involved but experiments on the effects of these glands on plasma inorganic phosphate are suggested.

(10) The secondary fall of plasma inorganic phosphate below the pre-injection level after injection of anterior pituitary extract may be due to a reflex stimulation of insulin secretion by the hyperglycaemia, or an effect of the extract on the thyroid or parathyroids evoking a secretion of thyroid or parathyroid hormone.

(11) A direct endocrine relationship between the anterior pituitary and plasma inorganic phosphate is suggested.

(12) Injection of potassium phosphate in amounts equal to the total blood inorganic phosphate content of a toad produces no effect on the plasma inorganic phosphate whereas injection of 0.5 ml fluid produces a slight diluting effect on the plasma.

THE EFFECT OF THE PARATHYROIDS ON THE PLASMA INORGANIC PHOSPHATE.

D. THE PARATHYROID GLANDS.

PARATHYROIDECTOMY.

The anatomy and topography of the parathyroid glandules in Xenopus laevis have been described by Shapiro (1933a). The glandules lie very deeply between the carotid arch anteriorly, the systemic arch posteriorly and the hypoglossal nerve medially and are so small as to be invisible to the naked eye. As it is almost impossible to remove the parathyroids without damaging the main systemic vessels no attempt at parathyroidectomy was made.

(a) Injection of parathyroid extract into pond females.

Fresh pond females were kept in the laboratory for one week before injection. The parathyroid extract used was a commercial preparation "Paroidin" manufactured by Parke, Davis and Co.

In the first batch of toads used the injections were given intraperitoneally. In the second batch they were made by the ventral lymphatic route. In every case each animal was injected with 0.25 ml. of the preparation which was equivalent to 25 Hanson units. Controls were injected with frog Ringer solution via the same route and in the same quantities as the parathyroid injected animals.

In all 20 dozen animals were used.
In Tables XV and XVI below the results of parathyroid injection on the plasma inorganic phosphate are shown.

TABLE XV.

Plasma inorganic phosphate after intraperitoneal injection of "Paroidin".

Pre-injection level 4.7 ± 0.17 (6)

Time in hours after injection	"Paroidin" 25 units	Ringer Soln. Control.
6	4.1 ± 0.16 (5)	
18	5.6 ± 0.14 (6)	4.6 ± 0.13 (4)
24	6.6 ± 0.15 (6)	
36	6.5 ± 0.17 (6)	
48	6.1 ± 0.20 (8)	5.3 ± 0.09 (3)
60	7.2 ± 0.21 (6)	
72	6.8 ± 0.15 (6)	5.2 ± 0.16 (2)

Approximately two toads were used for each estimation. Detailed figures appear in the appendix:protocol No. 11.

TABLE XVI. .....

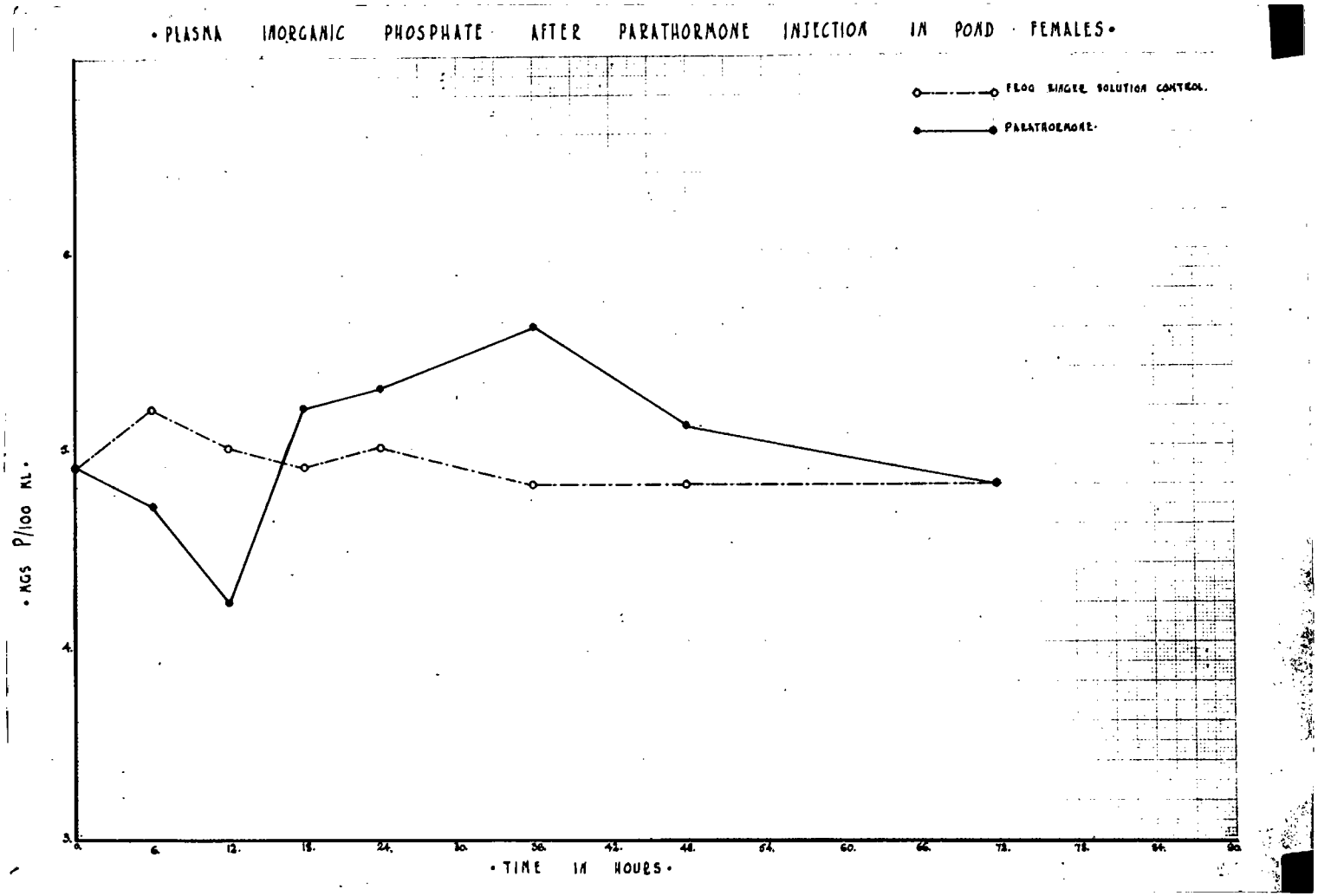

• PLASMA INORGANIC PHOSPHATE AFTER PARATHORMONE INJECTION IN POND FEMALES •
FROG RINGER SOLUTION CONTROL.
PARATHORMONE.
• MGS P/100 ML •
6.
5.
4.
3.
0.
6.
12.
18.
24.
30.
36.
42.
48.
54.
60.
66.
72.
78.
84.
90.
• TIME IN HOURS •

TABLE XVI.

Plasma inorganic phosphate after injection of "Paroidin" into the ventral lymph sac.

Pre-injection level 4.9 ± 0.24 (5)

Time in hours after injection	"Paroidin" 25 units	Ringer Soln. Control.
6	4.7 ± 0.16 (6)	5.2 ± 0.22 (4)
12	4.1 ± 0.05 (5)	5.0 ± 0.12 (5)
18	5.2 ± 0.12 (6)	4.9 ± 0.24 (6)
24	5.3 ± 0.06 (5)	5.0 ± 0.08 (7)
36	5.6 ± 0.14 (5)	4.8 ± 0.13 (5)
48	5.0 ± 0.14 (3)	4.8 ± 0.14 (4)
72	4.8 ± 0.11 (5)	4.8 ± 0.11 (3)

Each estimation required approximately 1.5 toads. Detailed figures appear in the appendix:protocol No. 12.
The results shown in Table XVI are graphically represented below (Graph VII).

(b) Repeated ....

(b) Repeated injection of parathyroid extract.

Four dozen toads from the batch which was used for intralymphatic injection of parathyroid hormone (Table XVI) were divided into three groups. The animals of the first group were controls and the pre-injectio plasma inorganic phosphate level was estimated on them. Animals of the second group (16) were injected intraperitoneally with 0.25 ml. "Paroidin" (25 Hanson units) daily for six consecutive days (i.e. 150 Hanson units parathyroid hormone in toto). The third group (16) each received 0.25 ml. frog Ringer solution intraperitoneally daily for six days. All the injections were given at the same hour each day. Animals were kept in a room artificially warmed to 21°C throughout the duration of the experiment. Estimations of plasma inorganic phosphate were made 24 and 48 hours after the last injection.

TABLE XVII

Plasma inorganic phosphate after repeated injections of "Paroidin"

Pre-injection level 4.9 ± 0.24 (5)

Time in hours after injection	"Paroidin 150 units	Ringer Soln. Control.
24	6.1 ± 0.14 (6)	4.9 ± 0.25 (4)
48	5.2 ± 0.37 (6)	5.2 ± 0.34 (3)

Detailed figures appear in the appendix:protocol No. 13.

DISCUSSION. ....

DISCUSSION.

Injection of parathyroid extract into normal toads.

Intraperitoneal or intralymphatic injection of parathyroid hormone produced the same effects namely an initial fall in plasma inorganic phosphate within the first 12 hours followed by a rise above the pre-injection level. The secondary rise of plasma inorganic phosphate is probably an overdosage phenomenon (see introduction) as a comparatively large dose of drug was injected. It will be observed in Table XV that the secondary rise of plasma inorganic phosphate was well marked whereas in Table XVI it was far less well shown. This is probably not due to the fact that the extract was injected by different routes but is probably due to an increased sensitivity of the first batch of animals to parathyroid hormone. The first experiment was carried out in autumn when the plasma inorganic phosphate is still high and as previously suggested the parathyroids may be hypo-functioning at this time. The second experiment was carried out two months later at the beginning of the breeding season when the parathyroids may be hyperfunctioning as previously discussed. It is possible that animals injected during the breeding season are more capable of dealing with additional parathyroid than animals injected before the breeding season, by virtue of the fact that the animals' own parathyroids

are .....

are over-functioning. It is well known that an animal becomes resistant to the effects of parathyroid injection after some time (Thomson and Collip, 1932; Pugsley and Collip, 1936).

The difference in plasma inorganic phosphate following "Paroidin" injection Table XVI and Graph VII are significant. The controls showed very little change from the pre-injection level, showing values of 5.2, 5.0, 4.9, 5.0, 4.8, 4.8, 4.8. The average figure is 5.0 0.06. In Table XVII below, the significant differences of the results, calculated as previously described, is shown.

TABLE XVII.

The significance of the figures showing changes in plasma inorganic phosphate following parathyroid hormone injection into the ventral lymph sac.

Average Control 5.0 ±0.06 (34)

Time in hours	6	12	18	24	36	48	72
Plasma inorganic phosphate	4.7	4.2	5.2	5.3	5.6	5.1	4.8
Significant difference	10:1	>100:1	3:1	100:1	>100:1	-	-

Mode .....

Mode of action of parathyroid hormone.

A single injection of parathyroid hormone into toads produced an initial fall in plasma inorganic phosphate with a subsequent rise above the pre-injection level. The possible modes of action have been discussed in the introduction and are as follows:-

(1) A mobilising action on the inorganic constituents of bone.

(2) A direct action on the renal threshold for phosphate.

The results found with Xenopus laevis cannot be taken to throw any further light on this particular problem and may support either hypothesis. The fact that the fall in plasma inorganic phosphate occurs very shortly after injection. (Table XV) may possibly favour the hypothesis that the primary action of parathormone is on the kidney rather than on the bones. Unfortunately no studies on serum calcium following parathyroid hormone injection have as yet been made on Xenopus. If the hypothesis of Albright and Ellsworth (1929) is adopted then the changes following parathyroid hormone injection might be interpreted in this way. Following injection of parathyroid hormone there is an immediate outpouring of phosphate in the urine and consequently the plasma inorganic phosphate falls. As the plasma inorganic phosphate falls the serum calcium rises and when the serum calcium reaches a critical level, the inorganic

phosphate .....

phosphate begins to rise. Finally both calcium and inorganic phosphate are above the pre-injection level. There is no permanent damage done to the kidney by the dose of the drug administered and the quantity is insufficient to maintain the hyperphosphataemia. As a result the plasma inorganic phosphate returns to the normal level.

The normal physiological effect of parathyroid hormone is probably to produce a fall in plasma inorganic phosphate. The subsequent rise probably only occurs with pathological hyperfunction.

In a previous section it was suggested that sexual variations and captivity effects, may be due to changes in parathyroid function. The results of parathyroid hormone injection on the plasma inorganic phosphate found are not in conflict with this view as the primary function of parathyroid is to produce a fall in plasma inorganic phosphate. The rise is probably a secondary effect.

The effect of hypophysectomy on plasma inorganic phosphate cannot be due to hypoparathyroidism as the parathyroids depress the plasma inorganic phosphate (this will be discussed further in the sequel). Injection of anterior pituitary extract produces a rise then a fall in plasma inorganic phosphate. It is possible that the secondary fall is due to stimulation of the parathyroids

Repeated .....

Repeated injection of parathyroid extract.

Twenty four hours after the last injection, the plasma inorganic phosphate was raised by 22 per cent. Within 48 hours, however, it had returned to the normal level. The animals showed no toxic signs and evidently the parathyroid hormone had done little damage. A few serial sections of kidneys were made but no signs of metastatic calcification nor of renal damage were observed.

These findings are in keeping with the work of Thomson and Collip (1932) who found that the plasma inorganic phosphate at first declines, but later increases and if the injections are continued the blood inorganic phosphate continues to rise steadily, throughout the experiment.

Cessation of the injection leads to a return to the normal level.

These results are the effects of large doses of parathyroid hormone given repeatedly and are not strictly comparable with normal physiological effects.

SUMMARY.

(1) Intraperitoneal or intralymphatic injection of parathyroid extract into normal female toads produces an initial fall then a rise in plasma inorganic phosphate above the pre-injection level.

(2) Repeated injections of parathyroid extract produce an elevated plasma inorganic phosphate level which returns to normal 48 hours after the cessation of injections.

(3) The normal physiological action of the parathyroids is probably to produce a fall in plasma inorganic phosphate. The subsequent rise is probably an overdosage phenomenon.

(4) Seasonal variations and captivity effects have been ascribed to changes in parathyroid activity. Results of experiments on the effect of parathyroid extract on plasma inorganic phosphate are in keeping with this hypothesis.

(5) The fall in plasma inorganic phosphate after hypophysectomy is probably not due to hypoparathyroidism as the latter would probably produce a directly opposite condition. Experiments showing the effect of parathyroid on hypophysectomised animals are required to substantiate the argument and this is discussed in the sequel.

(6) The secondary fall of plasma inorganic phosphate consequent on injection of anterior pituitary extract may possibly be due to stimulation of the parathyroid glands by the extract with the discharge of parathyroid hormone into the blood-stream.

(7) No further light has been thrown on the actual mode of action of parathyroid extract.

THE EFFECT OF THE GONADS ON THE PLASMA INORGANIC PHOSPHATE.

E THE GONADS.

(a) GONADECTOMY.

Fresh pond animals were gonadectomised in July. This month was chosen because the breeding season begins in this month and the ovaries are well developed. It was considered that any ovarian hormones secreted would be produced in the greatest quantity during the breeding season. If ovarian factors influence the level of the plasma inorganic phosphate at all, effects of ovariectomy would be well marked if the operation were performed when the gonads were acting at their maximum. It has been shown that the plasma inorganic phosphate level is lowest during the breeding season and the hypothesis put forward was that this may be due to parathyroid or ovarian activity.

Estimations were made at monthly intervals and the method of storage and feeding of animals was as described elsewhere.

In Table XVIII below the effects of ovariectomy on the plasma inorganic phosphate is shown.

TABLE XVIII ...

TABLE XVIII.

Plasma inorganic phosphate after ovariectomy.

Preoperative normal 4.7 ± 0.08 (11)

Time in months after operation	Ovariectomy	Op. Control
1	5.5 ± 0.41 (7)	5.4 ± 0.22 (3)
2	5.3 ± 0.15 (10)	5.3 ± 0.22 (8)
3	5.1 ± 0.08 (9)	5.4 ± 0.09 (8)
4	5.4 ± 0.15 (7)	5.7 ± 0.19 (5)
5	5.1 ± 0.19 (5)	5.0 ± 0.29 (4)
6	4.1 ± 0.28 (4)	4.1 ± 0.19 (4)

Each estimation required 1.5 toads. Detailed figures appear in the appendix:protocol No. 14.

Table XIX shows the acute effect of ovariectomy shortly after operation.

TABLE XIX.

Plasma inorganic phosphate 24 hours after ovariectomy.

Ovariectomy	Operative Control
6.4 (3)	6.3 (3)

Each estimation required approximately 3 toads. Detailed figures appear in the appendix:protocol No. 15.

In .....

In Table XX the effects of orchidectomy on plasma inorganic phosphate are shown.

TABLE XX.

Plasma inorganic phosphate following orchidectomy.

Time in months after operation	Castration	Op. Controls
1	4.4 ± 0.10 (3)	4.6 ± 0.24 (4)
3	4.8 ± 0.26 (3)	4.7 ± 0.12 (4) *

* Captivity control

Each estimation required 2 animals. Detailed figures appear in the appendix:protocol No. 15.

After bleeding the animals a post-mortem examination was made in every case to ascertain whether the gonadectomy had been complete. In every gonadectomised animal complete removal was effected. In the operative control animals, the ovaries remained in good condition for the first four months after which retrogressive and atrophic changes were noted.

Injection of mammalian ovarian extracts

Fresh pond animals were kept about a week before being used for injection. Mammalian ovarian extracts (crystalline oestradiol benzoate and progesterone) were used and for controls injections of nut oil were made. All injections were

administered ....

administered intraperitoneally and animals were kept in a room artificially warmed to about 20°C throughout the experiment as the experiments were performed early in winter. Progesterone produces ovulation and oviposition in Xenopus (Zwarenstein, 1936; Shapiro, 1936). During the first trial experiments smaller doses of progesterone were given and animals were kept at room temperature. It was found that ovulation did not occur. Animals were then kept in an artificially warmed atmosphere and graduated doses of progesterone administered. It was found that animals did not ovulate until doses of 500 µg. were given. The animals were thus peculiarly resistant to the action of progesterone at this time of the year (May) for from June onwards doses as small as 50 µg produce ovulation. Shapiro (1939c) found similar results.

Sixty four animals each received 250 µg. oestradiol benzoate in 0.1 ml. oil, 64 each received 500 µg. progesterone in 0.1 ml. oil and 65 each received 0.1 ml. nut oil. The progesterone was administered undiluted as obtained (5 µg. per ml.), the oestradiol benzoate was diluted with equal quantities of nut oil (5 µg. per ml.). Very accurately graduated 1.0 ml syringes were used for the injection (Wilmrod 1.0 ml. syringe graduated in 0.01 ml.).

Only the animals injected with progesterone ovulated. This is in accordance with the experiments described by

Zwarenstein ....

Zwarenstein, (1936), Shapiro (1936) and Berk (1939a). Oestradiol benzoate produced no macroscopical changes in the cloacal labia. This confirms the work of Shapiro and Zwarenstein (1937) who found no change macroscopically or microscopically (Berk, 1939a) after injections of oestradiol benzoate. The peritoneum was found to be hyperaemic in all animals and this is readily explicable by the fact that the vessels are dilated as a result of increased absorption from the peritoneum.

Ovulation counts were carried according to the method described by Zwarenstein (1937). This consists of assigning arbitrary symbols to the degree of ovulation 1 or 2 according to the number of eggs in the oviduct, 3 or 4 according to the number in the para uteri; and 5 or 6 according to the number of eggs extruded from the cloaca into the water in the container. The total score of all positive animals divided by the number of positive animals gives a figure called the ovulation index. When less than 10 per cent of animals ovulate, not much significance can be attached to the index. The maximum ovulation index is 6.

Changes in plasma inorganic phosphate following progesterone are given in Table XXI. In Graph VIII the effects of both progesterone and oestradiol benzoate are plotted together.

TABLE XXI .....

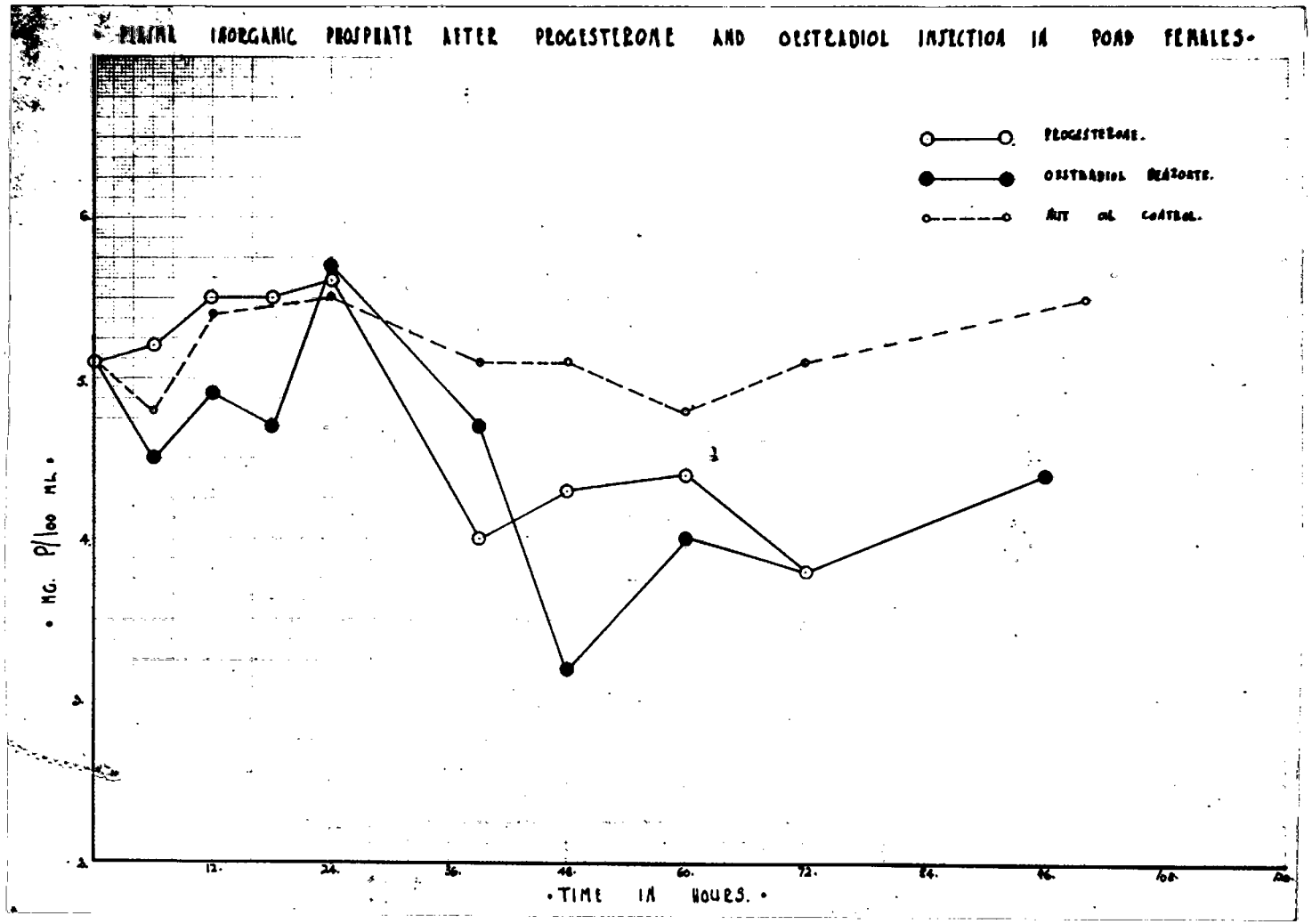

PLASMA INORGANIC PHOSPHATE AFTER PROGESTERONE AND OESTRADIOL INJECTION IN POND FEMALES.
PROGESTERONE.
OESTRADIOL BENZOATE.
OIL CONTROL.
MG. P/100 ML.
TIME IN HOURS.

CHART VII.

TABLE XXI.

Plasma inorganic phosphate following injections of progesterone.

Pre-injection level 5.1 ± 0.03 (6)

Time in hours after injection	Progesterone 500 µg.	Oil Control	Ovulation Index	
6	5.1 ± 0.34 (5)	4.8±0.09 (3)	1/1	
12	5.5 ± 0.37 (3)	5.4 (1)	1/1	
18	5.5 ± 0.27 (5)		11/5	2
24	5.6 ± 0.22 (4)	5.5±0.25 (2)	34/7	5
39	4.1 ± 0.04 (3)	5.1±0.40 (3)	19/6	3
48	4.3 ± 0.05 (5)	5.1±0.11 (5)	31/6	5
60	4.5 ± 0.15 (4)	4.8±0.12 (4)	26/7	4
72	3.8 ± 0.28 (4)	5.1±0.21 (4)	40/7	6
96	4.4 ± 0.20 (3)	5.5±0.18 (3)	4/1	

Each estimation required 2 toads. Detailed figures appear in the appendix:protocol No. 16.

TABLE XXII.

TABLE XXII.

Plasma inorganic phosphate following injections of oestradiol benzoate.

Pre-injection level 5.1 ± 0.03 (7)

Time in hours after injection	Oestradiol benz. 250 µ g.	Oil Control
6	4.5 ± 0.09 (7)	4.8 ± 0.09 (3)
12	4.9 ± 0.18 (3)	5.4 (1)
18	4.7 ± 0.18 (4)	
24	5.7 ± 0.10 (6)	5.5 ± 0.25 (2)
39	4.7 ± 0.12 (4)	5.1 ± 0.40 (3)
48	3.2 ± 0.12 (5)	5.1 ± 0.11 (5)
60	4.0 ± 0.44 (3)	4.8 ± 0.12 (4)
72	3.8 ± 0.16 (4)	5.1 ± 0.21 (4)
96	4.4 ± 0.12 (5)	5.5 ± 0.18 (3)

Each estimation required 2 toads. Detailed figures appear in the appendix:protocol No. 17.

Graph VIII ...

DISCUSSION.

Gonadectomy.

Tables XVIII, and XX reveal that gonadectomy produces no change in plasma inorganic phosphate up to 6 months (females) and 3 months (males) after operation. The plasma inorganic phosphate remained remarkably steady, figures for ovariectomy being:-

(4.7, i.e. preoperative normal), 5.5, 5.3, 5.1, 5.4, 5.1, 4.1

Operative controls showed the following figures:-

(4.7, i.e. preoperative normal), 5.4, 5.3, 5.4, 5.7, 5.0, 4.1.

No change in plasma inorganic phosphate was observed 24 hours after ovariectomy (Table XIX).

Injection of progesterone and oestradiol benzoate.

Tables XXI and XXII and graph VIII reveal that both progesterone and oestradiol benzoate produce a fall in plasma inorganic phosphate 48 hours after injection. The injected controls showed little variation the values being:-

5.1, 4.8, 5.4, 5.5, 5.1, 5.1, 4.8, 5.1, 5.5.

In Table XXIII below the significant differences of the results, calculated as previously described is shown.

TABLE XXIII .....

TABLE XXIII.

The significance of figures showing changes in plasma inorganic phosphate following progesterone and oestradiol benzoate injection in pond females.

Average Control 5.1 ± 0.06 (32)

Time in hours after injection	6	12	18	24	39	48	60	72	96
Progesterone		5.3	5.5	5.6	4.1	4.3	4.5	3.6	4.4
Oestradiol Benzoate	4.5	4.9	4.7	5.7	4.6	3.2	4.0	3.6	4.4
Significant Difference Progesterone		-	-	0	20:1	>100:1	5:1	100:1	>100:1
Significant Difference Oestradiol Benzoate	20:1	-	-	0	0	>100:1	10:1	>100:1	>100:1

Mode of .....

Mode of action of the gonads.

Gonadectomy had no effect on the plasma inorganic phosphate 6 months after operation in the case of females and 3 months in the case of males. In a previous section seasonal variations in plasma inorganic phosphate were described and it was found that during the breeding season, the plasma inorganic phosphate was significantly depressed. One of the possible explanations of this fall in plasma inorganic phosphate suggested, was that it was produced by ovarian secretion. It was also suggested that this might occur through the intermediation of the gonadotrophic substance of the anterior pituitary. Gonadectomy was performed at the height of the breeding season. Both acute experiments and experiments extending over 6 months showed that gonadectomy had no effect on the plasma inorganic phosphate. It is probable, therefore, that the gonads are not involved in the production of the seasonal changes in plasma inorganic phosphate.

It is interesting to compare the effects of gonadectomy on the serum calcium with the effects in plasma inorganic phosphate. Shapiro (1933b) on Xenopus laevis found that ovariectomy led to a persistent fall in the serum calcium level but this effect was not observed immediately after removal of the glands. It first became apparent 2 weeks after ovariectomy. In males there was no effect even 3.5

months .....

months after orchidectomy but the typical male level was reached by female toads 3.5 months after removal of the ovaries. Males showed a fall in serum calcium level after 6 months castration. Since the gonadectomy effects were not observed immediately after removal of the gonads it appears likely that other endocrine agencies are also involved in bringing about the changes in calcium metabolism described e.g. the pituitary.

If the pituitary is a contributary factor in the production of the fall in serum calcium level an explanation of the atypical behaviour of plasma inorganic phosphate may be given. It has previously been shown that as a rule serum calcium varies in inverse proportion to the plasma inorganic phosphate. In the case of the anterior pituitary, however, this relation does not occur. Both serum calcium and plasma inorganic phosphate show a fall after hypophysectomy. The fall in serum calcium is attributed to the loss of the pars tuberalis (Shapiro, 1933b) since the serum calcium level returns to normal when the pars tuberalis regenerates. Plasma inorganic phosphate, however, does not return to normal when the pars tuberalis regenerates. It is probable that calcium and phosphorus metabolism are affected by different pituitary factors. Castration produces a fall in serum calcium but no change in plasma inorganic phosphate. It is possible that the pituitary factor affecting calcium metabolism is involved and a rise in plasma inorganic phosphate is thus not a neces-

sary .....

eary concomitant event.

Injection of oestradiol benzoate and of progesterone produce a fall in plasma inorganic phosphate. This fall in plasma inorganic phosphate occurs independent of ovulation and oviposition as it occurs with oestradiol benzoate which has no effect on the ovary or accessory sexual organs (Shapiro and Zwarenstein, 1937; Berk, 1939a). In Table XXI the fall in plasma inorganic phosphate occurs when oviposition is at its maximum. This finding is not consistent, however, and subsequent experiments showed no correlation between oviposition, ovulation and plasma inorganic phosphate. In some experiments the fall in plasma inorganic phosphate occurred before oviposition and in others the reverse.

Shapiro (1933b) found that active extracts of ovarian tissue significantly raised the lowered level of serum calcium in castrated female toads and also raised the level of serum calcium in normal toads above the normal level. There is evidence, however, that the principles exerting a hormone-like action on calcium metabolism are neither oestrogenic preparations nor corpus luteum extracts. In the case of the pituitary, the gonadotrophic hormones are not a likely stimulus. Here again the effect on calcium and phosphorus differs. It is possible that oestradiol and progesterone produce changes in plasma inorganic phosphate

by .....

by affecting the intermediary metabolism of phosphorus. Both these substances contain the physiologically important cyclo-pentanophenanthrene nucleus. It is also probable, however, that the animal's own ovaries do not affect phosphorus metabolism. The action of mammalian ovarian extracts is not strictly comparable with the action of amphibian ovarian extract. Experiments are required to show the effects of Xenopus ovarian extract on plasma inorganic phosphate before formulating any definite conclusions. Available evidence points to the fact that mammalian and amphibian gonadal hormones are not identical. Intralymphatic or intraperitoneal injection of mammalian androgens into male Xenopus and of mammalian oestrogens into females did not result in amplexus. The experiments were done on anoestrous animals and repeated on gonadectomised animals. There was no hyperaemia of the cloacal labia in the females. This is not a question of absorption as the succinate and other esters of male hormone dissolved in propylene glycol still gave negative results (Shapiro, 1940). Progesterone is gonadotrophic in Xenopus producing no labial reaction in the ovariectomised female (Shapiro, 1940). Berk (1939b) showed that methyl testosterone, testosterone proprionate, oestradiol benzoate and progesterone were not androgenic in male Xenopus. These substances include mammalian androgens which should have stimulated nuptial pad development in male Xenopus.

SUMMARY .....

SUMMARY.

(1) Gonadectomy produces no change in plasma inorganic phosphate 6 months after operation in the case of females and 3 months in the case of males. Serum calcium, however, shows a fall 2 weeks after ovariectomy and 6 months after orchidectomy.

(2) It is probable that the change in calcium is affected partly by the anterior pituitary. Serum calcium and plasma inorganic phosphate are probably affected by different anterior pituitary principles.

(3) Seasonal variations in plasma inorganic phosphate are probably not due to ovarian activity.

(4) Injection of oestradiol benzoate and of progesterone produces a fall in plasma inorganic phosphate. Ovarian extracts produce a rise in serum calcium but the responsible factor is probably neither oestrin nor progesterone.

(5) It is probable that the ovary in the amphibian has no effect on the plasma inorganic phosphate though it may affect the serum calcium either directly or indirectly.

(6) It is probable that the effect of mammalian extract on plasma inorganic phosphate is not strictly comparable with the effect of the animal's own ovaries on phosphorus metabolism. The amphibian ovary probably produces hormones which are not identical with the mammalian hormones, oestradiol and progesterone.

THE EFFECT OF THE THYROID ON PLASMA INORGANIC PHOSPHATE.

THE THYROID GLAND.

(a) Thyroidectomy

Thyroidectomised and operative control animals were prepared as described elsewhere. Five months and later after operation animals that had been kept in captivity for the same length of time and from the same original batch as the thyroidectomised animals were used for operative controls.

Estimations were made at monthly intervals for 7 months, the storage and feeding being as described. A number of post-mortem examinations was made after bleeding thyroidectomised and operative control animals and a macroscopical examination for the thyroid glands was made. No thyroid tissue was seen in the thyroidectomised animals but serial sections of the tissues in the region of the thyroid glands were not cut.

It was observed after 5 months that thyroidectomised animals showed marked emaciation. The controls also definitely lost weight but this was not as great as in the thyroidectomised animals. Gitlin (1939c) has found similar results.

In all about 5 dozen thyroidectomised and the same number of operative controls were used.

Table XXIV shows the effect of thyroidectomy on plasma inorganic phosphate.

TABLE XXIV .....

GRAPH IV.

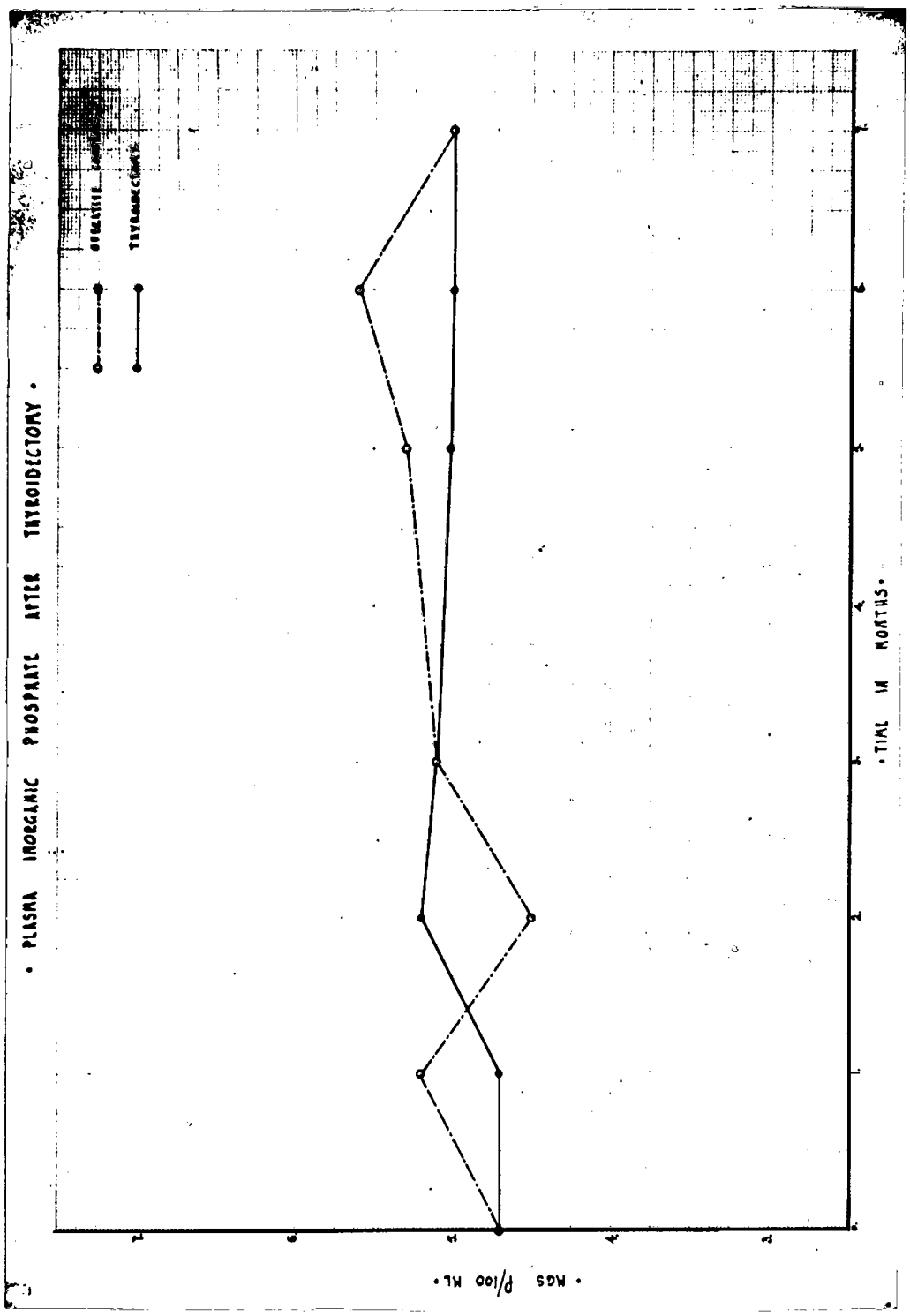

• PLASMA INORGANIC PHOSPHATE AFTER THYROIDECTOMY •
OPERATIVE
THYROXINE
• MGS P/100 ML •
• TIME IN MONTHS •

TABLE XXIV.

Plasma inorganic phosphate following thyroidectomy

Pre-injection level 4.7 ± 0.17 (6)

Time in months after operation	Thyroidectomy	Operative Controls
1	4.7 ± 0.15 (3)	5.2 ± 0.44 (3)
2	5.2 ± 0.28 (6)	4.5 ± 0.21 (5)
3	5.1 ± 0.11 (6)	5.1 ± 0.18 (5)
4		
5	5.0 ± 0.13 (8)	5.3 ± 0.26 (5) *
6	5.0 ± 0.23 (7)	5.6 ± 0.22 (5) *
7	5.0 ± 0.20 (4)	5.0 ± 0.27 (4) *

* Captivity control

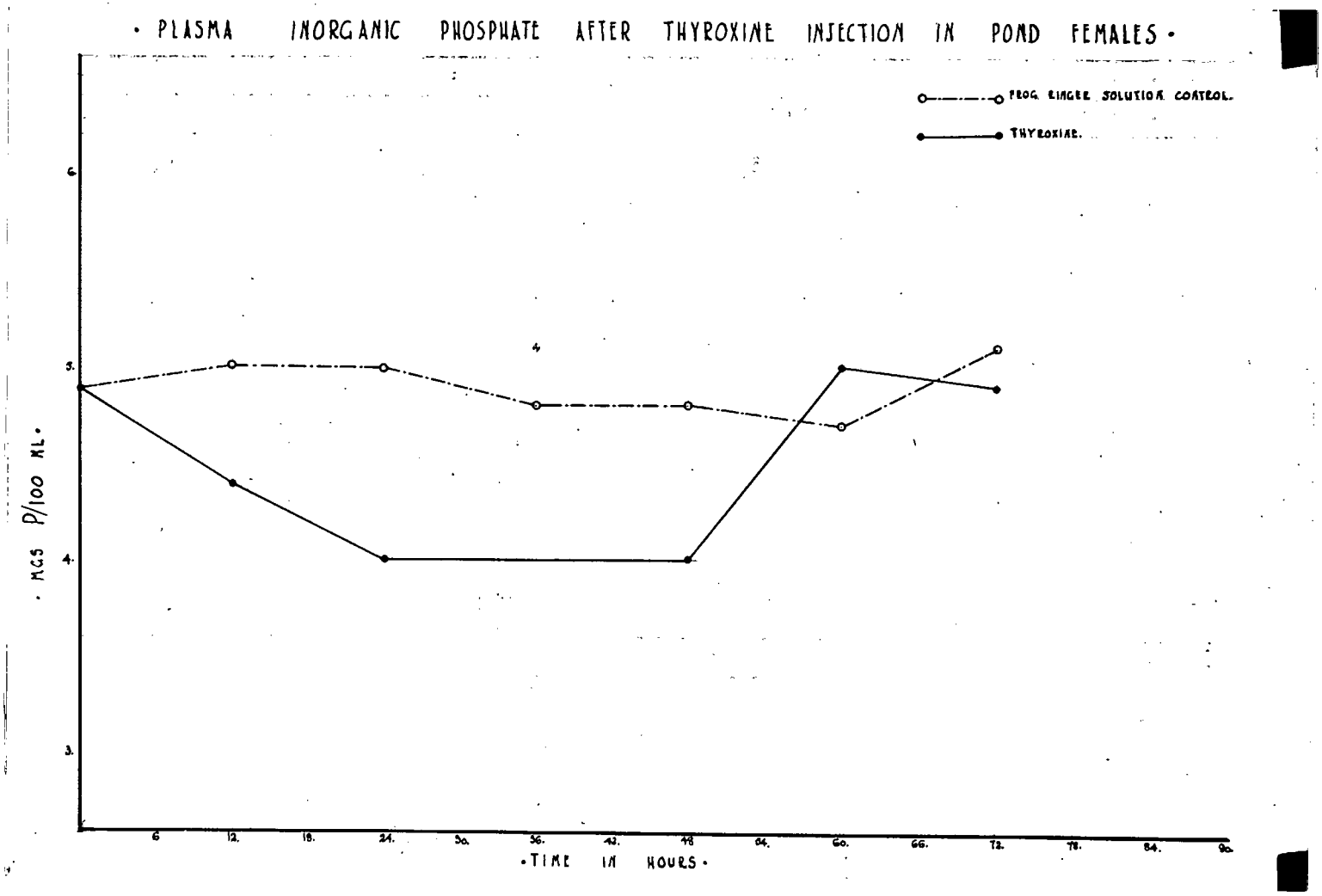
Each estimation required 1.5 animals. The detailed figures appear in the appendix:protocol No. 19.

The results of Table XXIV are graphically represented below (Graph IX).

(b) Injection of sodium thyroxine into normal females

Fresh pond females were kept for a week in the laboratory before being used for injection.

Each animal was injected with 0.1 mg thyroxine sodium (B.D.H.) ....


• PLASMA INORGANIC PHOSPHATE AFTER THYROXINE INJECTION IN POND FEMALES •
FROG RINGER SOLUTION CONTROL.
THYROXINE.
• MGS P/100 ML •
6
5
4
3
6
12.
18.
24.
30.
36.
42.
48
54.
60.
66.
72.
78.
84.
90.
• TIME IN HOURS •

(B.D.H.) in 0.25 ml frog Ringer solution. Controls received the same amount of frog Ringer solution. Parathyroid and thyroid injections were given simultaneously so that the same controls could be used for both hormones (see parathyroid injection).

All injections were given into the ventral lymph sac and animals were kept at room temperature (19°C). The results obtained are shown below (Table XXV).

TABLE XXV.

Plasma inorganic phosphate of pond females injected with thyroxine sodium

Pre-injection level 4.9 ± 0.24 (5)

Time in hours after injection	Thyroxine Sodium	Ringer Solution Control
12	4.4 ± 0.12 (5)	5.0 ± 0.12 (5)
24	4.0 ± 0.11 (5)	5.0 ± 0.08 (7)
48	4.3 ± 0.26 (4)	4.8 ± 0.14 (4)
60	5.0 ± 0.12 (4)	4.7 ± 0.07 (3)
72	4.7 ± 0.28 (5)	5.1 ± 0.25 (4)

Each estimation required approximately 3 toads. Detailed figures appear in the appendix:protocol No. 20.

The above results are graphically represented below. (Graph X)

(c) Repeated .....

(c) Repeated injection of thyroxine sodium

A batch of female toads was kept in the laboratory for about one week before being injected. They were then divided into three groups the first group being killed and the plasma inorganic phosphate estimated. This gave the preinjection value.

The second group (17) were injected with daily doses of 0.1 mg. sodium thyroxine in 0.25 ml. frog Ringer solution for 6 days, (Ciba Ltd. thyroxine sodium) i.e. 0.6 mg. in all. The remaining group received 0.25 frog Ringer solution daily for 6 days. All injections were given intraperitoneally and the animals were kept in an artificially warmed room throughout the experiment. The toads were killed off 24 and 48 hours after the last injection.
The animals were not fed during the experiment.
The results of this experiment will be found in Table XXVI below.

TABLE XXVI.

Plasma inorganic phosphate of fresh pond females injected for 6 days with thyroxine sodium.

Time in hours after injection	Thyroxine sodium	Ringer Solution Cont.
24	6.0 ± 0.24 (5)	4.9 ± 0.25 (4)
48	6.1 ± 0.23 (6)	5.2 ± 0.34 (3)

Each .....

Each estimation required 2 toads. Detailed figures appear in the appendix:protocol No. 21.

This experiment was repeated, injections of sodium thyroxine being given daily for 4 days. Controls were injected with nut oil 0.1 ml per injection for 4 days. The results are shown in Table XXVII.

TABLE XXVII.

Plasma inorganic phosphate of fresh pond females injected for 4 days with thyroxine sodium.

Time in hours after injection	Thyroxine sodium 0.4 mg	Nut oil control
24	5.2 ± 0.22 (4)	4.7 ± 0.09 (6)
48	5.4 ± 0.16 (5)	4.7 ± 0.15 (5)

Each estimation required 2 toads. Detailed figures appear in the appendix:protocol No. 21.

DISCUSSION .....

DISCUSSION.

Thyroidectomy

Table XXIV and Graph IX reveal that thyroidectomy produced no change in plasma inorganic phosphate up to 7 months after operation. The plasma inorganic phosphate remained remarkably steady, figures for thyroidectomy being:- 4.7, 5.2, 5.1, 5.0, 5.0, 5.0; and for operative controls 5.2, 4.5, 5.1, 5.3, 5.6, 5.0.

Injection of thyroxine sodium

Table XXV and Graph X reveal that the injection of thyroxine sodium produces an initial fall of plasma inorganic phosphate with a subsequent return to the normal level. A fall of 20 per cent is recorded 24 hours after injection and the normal level is reached in 60 hours.

The injected controls showed little variation the values being:- 5.0, 5.0, 4.8, 4.8, 4.7, and 5.1 with an average of 4.9 0.06 (28).

The significant difference between thyroxine injected and control animals, calculated from the formula is shown below in Table XXVIII.

TABLE XXVIII.

The significant difference between thyroxine injected and control animals. Average Control 4.9 (28).

Time in hours after injection	12	24	48	60	72
thyroxine injected	4.4	4.0	4.3	5.0	4.9
significant difference	>100:1	>100:1	>10:1	-	-

This effect of thyroxine on the plasma inorganic phosphate has been confirmed in subsequent experiments (see sequel).

Repeated injections of thyroxine sodium

Table XXVI shows that after 6 injections of throxine (0.6 mg.) the plasma inorganic phosphate is raised 24 per cent. The plasma inorganic phosphate has not returned to the normal level 48 hours after cessation of injections.

Table XXVII shows that after 4 injections of thyroxine (0.4 mg.) the plasma inorganic phosphate is raised 15 per cent and has not returned to the normal level 48 hours after cessation of injections.

Mode of action of the thyroid

Thyroidectomy produces no change in the plasma inorganic phosphate of Xenopus laevis. This is in accord with the work of Gerschman and Marenzi on dogs (1935). There is a slight decrease or no effect on the serum calcium of thyroidectomised animals (see introduction). This means either that the thyroid has no direct effect on the calcium and inorganic phosphate concentrations of blood or that any effects it may have are masked by compensating mechanisms when it is removed.

A single injection of thyroxine produces a fall in inorganic phosphate. A possible interpretation of the mode of action may be the following:- With the administration of thyroxine the oxidative processes of the body are speeded

up ....

up, carbohydrates are oxidised and a demand for inorganic phosphate is set up to assist in phosphorylating the intermediary products of carbohydrate metabolism. At the same time calcium and phosphate are mobilised from the bones (Aub et al., 1929). The call on inorganic phosphate for intermediary metabolism is greater than the supply from the bones and tissues and as a result the plasma inorganic phosphate falls. It is interesting to note that there is a tendency for the calcium to rise with thyroid administration (see introduction).

Repeated injections of thyroxine produce a rise in plasma inorganic phosphate. After repeated injections of thyroid extract or thyroxine there is a slight hyperglycaemia and the glycogen reserves are mobilised. As a result the glycogen reserves gradually become depleted, carbohydrate is oxidised quicker than it can be replaced and eventually the animals may die in hypoglycaemia. In the latter stage of hypoglycaemia the call for inorganic phosphate is no longer as great, as there is no longer enough carbohydrate left to oxidise and in consequence the plasma inorganic phosphate may rise. This rise is enhanced by the fact that calcium and phosphate have been mobilised continuously from the bones by a direct action of the thyroid extract.

The fall in plasma inorganic phosphate following hypophysectomy cannot be ascribed to hypofunction of the thyroid

as .....

as thyroidectomy produces no change in plasma inorganic phosphate. This fact can be further substantiated by injecting thyroxine into hypophysectomised animals and ascertaining whether the plasma inorganic phosphate level is restored. This will be discussed in the sequel.

Similarly the rise in plasma inorganic phosphate following injection of anterior pituitary extract cannot be due to stimulation of the thyroid gland as thyroxine produces a fall. The secondary fall of plasma inorganic phosphate may be due to stimulation of the thyroid gland.

SUMMARY. ....

SUMMARY.

(1) Thyroidectomy produces no change in plasma inorganic phosphate up to 7 months after thyroidectomy.

(2) Injection of 0.1 mg. thyroxine produces a fall of plasma inorganic phosphate in normal animals with a subsequent return to the normal level.

(3) Repeated injection of thyroxine (total dose of 0.4 - 0.6 mg.) produces an elevated plasma inorganic phosphate level which does not return to the normal level within 48 hours after cessation of injections.

(4) Under physiological conditions the thyroid probably produces no change in plasma inorganic phosphate.

(5) The fall in plasma inorganic phosphate following injection of thyroxine is attributed to an increased demand for inorganic phosphate consequent on increased metabolic activity.

(6) The rise in plasma inorganic phosphate following repeated injections of thyroxine is attributed to consumption of carbohydrate stores with consequent diminished demand for inorganic phosphate. Mobilisation of calcium and phosphate from the bones and the soft tissues probably assists this reaction.

(7) The fall in plasma inorganic phosphate after hypophysectomy is probably not due to hypothyroidism as thyroidectomy produces no change. Experiments showing the effect of thyroxine on hypophysectomised animals are required to substantiate the argument and this is discussed in the sequel.

(8) The ....

(8) The secondary fall of plasma inorganic phosphate consequent on injection of anterior pituitary extract may possibly be due to stimulation of the thyroid glands with the discharge of thyroid hormone into the blood stream.

THE EFFECT OF THE PANCREAS ON PLASMA INORGANIC PHOSPHATE.

THE PANCREAS.

Pancreatectomy.

Pancreatectomy was performed as described. Animals did not survive pancreatectomy for longer than 2 - 3 days, so that acute effects of pancreatectomy were investigated. Ether produces a hyperglycaemia (Burn, 1915) and a hyperphosphataemia Marenzi and Gerschman (1934a) and this may affect the plasma inorganic phosphate of Xenopus laevis. In Table XXIX below the effect of ether 6 hours after the anaesthetic is shown.

TABLE XXIX.

Plasma inorganic phosphate 6 hours after ether anaesthesia in animals fresh from the pond.

Ether	Control
7.7 (5)	7.7 (6)

Each estimation required 2 toads. Detailed figures appear in the appendix:protocol No. 23.

From Table XXIX, it can be seen that ether has no effect on the plasma inorganic phosphate 6 hours after anaesthesia.

Pancreatectomised animals were estimated at various intervals from 3 - 48 hours after operation. Blood sugar estimations were made according to the Harding modification of Samogy's method (Harding and Downs, 1933). These values are true fermentable sugar values.

GRAPH XI.

PLASMA INORGANIC PHOSPHATE AND BLOOD SUGAR AFTER PANCREATECTOMY.
PANCREATECTOMY (PLASMA INORGANIC P).
OPERATIVE CONTROL (PLASMA INORGANIC P).
PANCREATECTOMY (BLOOD SUGAR)
OPERATIVE CONTROL (BLOOD SUGAR).
MG. P/100 ML.
MG. SUGAR/100 ML.
TIME IN HOURS.

In Table XXX below the effects of pancreatectomy on the plasma inorganic phosphate and blood sugar is shown.

In all approximately 8 dozen animals were pancreatectomised and 5 dozen operative controls were made.

TABLE XXX.

Plasma inorganic phosphate and blood sugar following pancreatectomy.

Pre-operative normal 6.3 ± 0.12 (5)

Time in hours after operation	Pancreatectomy		Operative control	
	Plasma inorganic Phos.	Blood sugar	Plasma inorganic Phos.	Blood sugar
3	8.0±0.29 (6)		6.6±0.23 (3)	
6	8.2±0.25 (7)	131 (7)	6.9±0.18 (5)	70 (6)
12	8.2±0.34 (8)	122 (5)	6.5±0.22 (4)	37 (4)
24	6.8±0.25 (8)	76 (6)	5.5±0.55 (2)	20 (4)
48	6.6±0.25 (4)	54 (2)	5.7±0.29 (3)	37 (4)

Each estimation required 3 toads. Detailed figures appear in the appendix:protocols No. 22

The figures for blood glucose are expressed in mgs./100 ml. Booman and Zwarenstein (1930) found that the blood sugar level of Xenopus laevis varied with the temperature but that at given temperatures the blood sugar level was remarkably constant. At temperatures of 5°C, 10°C and 20°C the blood sugar .....

sugar levels were 69, 57 and 44 respectively. In the above experiment, (Table XXX) the temperature varied from 19°C to 22°C.

The above results are graphically represented below. (Graph XI).

(b) Injection of insulin in pancreatectomised animals

Animals were pancreatectomised as previously described. The mortality was high and large numbers of animals died before injections were given. (The importance of post-operative care was only subsequently realised). Animals were injected with 2 units insulin contained in 0.25 ml. frog Ringer solution 6 hours after operation as it had previously been found (see Table XXX) that 3 - 12 hours after pancreatectomy the plasma inorganic phosphate is significantly raised. As controls pancreatectomised animals were injected with 0.25 ml. frog Ringer solution. All injections were given intraperitoneally. Probably no leakage occurred as the abdomen was sutured with a continuous suture which completely closed the wound. The results are shown in Table XXXI below.

Table XXXI ...

TABLE XXXI.

Plasma inorganic phosphate following insulin injection into pancreatectomised animals.

Pre-operative normal 5.1 (3)

Pancreatectomised (8 hours) Pre-injection level 6.9 (2)

Time in hours after injection	Pancreatectomised toads injected insulin	Pancreatectomised Ringer Solution Control
4	5.8 (2)	6.1 (1)
6	5.7 (2)	6.2 (2)
12	5.4 (2)	6.0 (2)

Each estimation required 4 toads. Detailed figures appear in the appendix:protocol No. 23.

(b) The effect of insulin injections on normal females.

At first 1 unit of insulin was injected intra-peritoneally in each toad. The insulin was injected in 0.5 ml. frog Ringer solution. No controls were made. Table XXXII below shows the effect of this injection on plasma inorganic phosphate and blood glucose.

The room temperature was 21°C.

Table XXXII ...

TABLE XXXII.

Plasma inorganic phosphate and blood glucose following injection of 0.5 ml. from Ringer solution containing 1 unit insulin.

Pre-injection level 6.9 (11)
Blood sugar 78 (1).

Time in hours after injection	Plasma inorganic P	Blood Glucose
1	5.6 (6)	56 (2)
2	4.5 (5)	42 (2)
3	3.9 (5)	
4	3.3 (4)	27 (2)
6	5.7 (5)	
12	5.7 (4)	

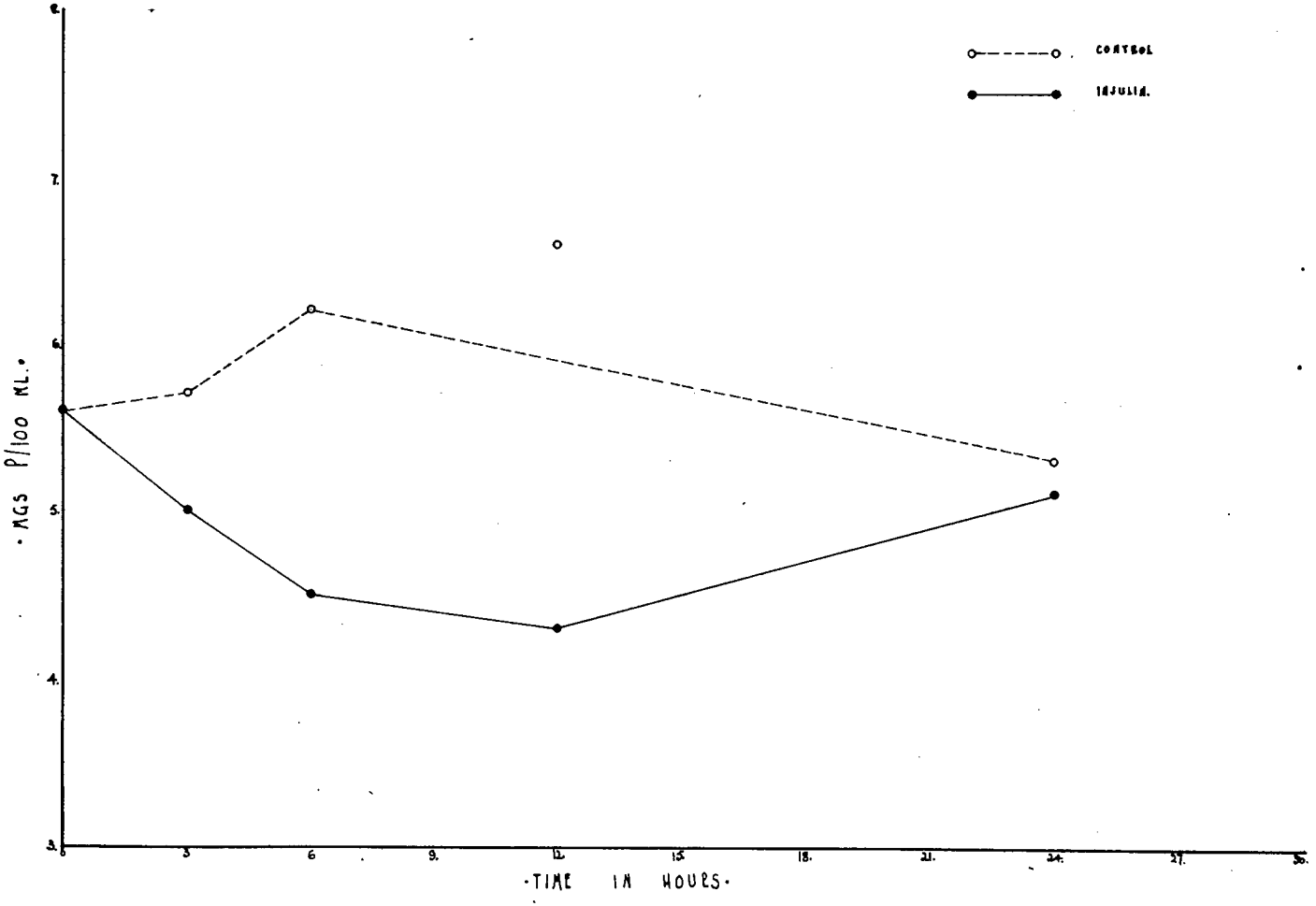
Each estimation required 2 animals. Detailed figures appear in the appendix:protocol No. 24.

Blood glucose is expressed in mg. per 100 ml. blood.

It was found, however, that 0.5 ml. fluid injected alone tends to produce a fall in plasma inorganic phosphate because of a diluting effect on the blood (c.f. anterior pituitary extract and phosphate injections) Two units of insulin contained in 0.1 ml. mammalian saline (undiluted Burroughs Wellcome crystalline insulin) was, therefore,

injected .....

∴ PLASMA INORGANIC PHOSPHATE AFTER INSULIN INJECTION IN POND FEMALES.



injected intraperitoneally into each animal. Controls received 0.1 ml. frog Ringer solution intraperitoneally. In all about 11 dozen animals were injected with insulin and the same number with frog Ringer solution.

Table XXXIII below shows the effect of 2 units insulin on the plasma inorganic phosphate.

TABLE XXXIII.

Plasma inorganic phosphate of pond females injected with 2 units insulin

Pre-injection level 5.6 ± 0.23 (3)

Time in hours after injection	Insulin 2 units	Ringer Solution Control.
3	5.0 ± 0.37 (4)	5.7 ± 0.0 (3)
6	4.5 ± 0.18 (4)	6.2 ± 0.20 (4)
12	4.3 ± 0.39 (4)	6.6 ± 0.09 (3)
24	5.1 ± 0.32 (5)	5.3 ± 0.07 (5)

Each estimation required approximately 2 animals. Detailed figures appear in the appendix:protocol No. 25.

The above results are graphically represented below (Graph XII).

In .....

In Table XXXIII it was noticed that an unaccountable rise of plasma inorganic phosphate occurred in the controls after 12 hours.

In Tables XXXIV, XXXV and XXXVI below in similar experiments this did not occur.

TABLE XXXIV.

Plasma inorganic phosphate following insulin injection (2 units).

Pre-injection level 6.7 (4)

Time in hours after injection	Insulin 2 units	Ringer Solution Control
6	4.9 (8)	5.8 (4)
24	5.2 (4)	5.9 (3)

In Tables XXXV and XXXVI below plasma inorganic phosphate 24 - 72 hours after injection of insulin is shown. In this series of injections the animals secreted large amounts of thick mucus from 50 hours and onwards after injection; several animals died. Some animals showing hypoglycaemic tremors were found.

Table XXXV ....

TABLE XXXV.

Plasma inorganic phosphate 24 - 50 hours after insulin injection

Pre-injection level 6.9 (3)

Time in hours after injection	Insulin 2 units	Ringer Solution Control
24	4.6 (5)	6.4 (4)
40	5.2 (5)	6.5 (4)
50	5.1 (3)	6.0 (3)

TABLE XXXVI.

Plasma inorganic phosphate 50 - 74 hours after insulin injection

Pre-injection level 5.2 (5)

Time in hours after injection	Insulin 2 units	Ringer Solution Control
50	6.4 (4)	5.1 (3)
64	6.5 (2)	5.4 (4)
74	6.3 (2)	5.8 (3)

In Tables XXXV and XXXVI each estimation required 3 animals. Detailed figures appear in the appendix:protocol No. 26.

Table .....

Table XXXVII records a similar experiment but the rise in plasma inorganic phosphate occurred 48 hours after injection.

TABLE XXXVII.

Plasma inorganic phosphate following insulin injection.

Time in hours after injection	Insulin 2 units	Ringer Solution Control
3	5.0 (4)	6.2 (4)
12	5.0 (4)	
48	7.4 (2)	5.9 (2)

Each estimation required approximately 2 animals. Detailed figures appear in the appendix:protocol No. 25.

Discussion .....

DISCUSSION.

(a) Pancreatectomy.

Inspection of Table XXX and Graph XI shows that pancreatectomy is followed by a rise in plasma inorganic phosphate. This was evident 3 hours after operation and reached a maximum after 6 - 12 hours, returning towards normal in 24 - 48 hours. Animals did not survive pancreatectomy longer than 48 hours. As compared with operative controls there was a maximum increase of 20 - 25 per cent. The controls showed a drop 24 hours after operation for some unknown reason the figures being 6.3, 6.6, 6.9, 6.5, 5.5, and 5.7. Only 2 estimations were made at 24 hours and it is probable that the control figure should be nearer 6.0 (see protocol No. 22). The average control figure is 6.3 ± 0.17 (17). The significant differences as calculated from the formula are shown in Table XXXVIII below.

TABLE XXXVIII.

The significant difference between pancreatectomised and control animals.

Average control 6.3 (17)

Time in hours after operation	3	6	12	24	48
Pancreatectomy	8.0	8.2	8.2	6.8	6.6
Significant difference	>100:1	>100:1	>100:1	20:1	20:1

In .....

In Table XXXI the effect of pancreatectomy is confirmed 6 hours after pancreatectomy plasma inorganic phosphate had risen from 5.1 to 6.9 i.e. a rise of 36 per cent. No operative controls were made, however.

It is interesting to note that the blood sugar showed analogous changes to the plasma inorganic phosphate. The maximum blood sugar values occurred when the plasma inorganic phosphate was maximum as shown below in Table XXXIX. The control blood sugar remained fairly constant being 44, 70, 37, 20 and 37. It is probable that temperature played no part in the production of these results. A difference of temperature of 15°C (5°C - 20°C, Bosman and Zwarenstein, 1930), produced a change of blood sugar of 25 mg./100 ml. (69 to 44) whereas pancreatectomy produced a rise of blood sugar up to 85 mg./100 ml. with temperature changes of 19°C to 22°C.

TABLE XXXIX ...

TABLE XXXIX.

Plasma inorganic phosphate and blood sugar following pancreatectomy.

Pre-operative levels 6.3 mg.P (5)
Blood sugar 45 (5)

Time in hours after operation	3	6	12	24	48
Plasma inorganic phosphate	8.0	8.2	8.2	6.8	6.6
Blood sugar		131	122	76	54

(b) Injection of insulin in pancreatectomised animals

The results of this experiment cannot be taken as too reliable as several samples of plasma were tinged with laked blood. However, the general tendency of the figures is to show a fall of plasma inorganic phosphate after injection of insulin. Pancreatectomy produces a rise. Although the figures are scanty there is no overlapping. (Table XXXI and protocol No. 23).

The results may be taken as contributory evidence as to the action of insulin and the pancreas on plasma inorganic phosphate.

(c) Injection of insulin

Inspection of Tables XXXII, XXXIII, XXXIV, XXXV and XXXVII ....

XXXVll and Graph Xll show that insulin produces a fall in plasma inorganic phosphate.

In Table XXXll 1.0 unit of insulin in 0.5 ml. solution was injected. Although this amount of fluid produces a fall in plasma inorganic phosphate this fall is 1.0 mg. at most. In this experiment a fall of over 100 per cent from 6.9 to 3.3 mg. was observed. It is probable that this fall is chiefly due to the action of insulin although dilution of the plasma may assist in this result. Again the close relationship between glucose and plasma inorganic phosphate is observed. This is tabulated below. (Table XL)

TABLE XL.

Plasma inorganic phosphate and blood sugar following insulin injection.

Time in hours after operation		1	2	3	4
Plasma inorganic phosphate	6.9	5.6	4.5	3.9	3.3
Blood sugar	75	56	42		27

The temperature remained constant so the effect observed cannot be ascribed to changes in temperature.

In Table XXXlll 2 units were given intraperitoneally and the experiment was controlled by injecting the same

amount ... ..

amount of frog Ringer solution. The controls showed an unaccountable rise in plasma inorganic phosphate which somewhat diminishes the value of the results. However, a steady fall of plasma inorganic phosphate can be seen in the insulin injected animals as follows 5.6, 5.0, 4.5, 4.3 with a return to the normal level in 24 hours. These results are graphically represented in Graph XII. In Table XXXIV the reverse is seen, namely, a fall in plasma inorganic phosphate of the controls. The fall, however, was much more marked in the insulin injected animals. The probable explanation of the unusual inconstancy of the controls is that these experiments were carried out early during the investigations before the importance of keeping animals for a week in the laboratory before using them, was realised.

In Table XXXV, it was found that the depressed inorganic phosphate level was maintained as long as 50 hours after injection.

A subsequent rise in plasma inorganic phosphate is observed in Table XXXVI. It is very interesting to study the protocol of this particular experiment (Protocol No. 26). This rise occurred 50 hours and more after injection. It was found that animals became semi-somatose about this time. Estimations made on the plasma from these semi-somatose animals showed a rise of up to 50 per cent above the level of the controls, whereas animals not somatose still showed

a .....

a fall. For example 50 hours after injection comatose animals showed inorganic phosphate values of 6.8, 8.2, and 6.4 respectively whereas non comatose animals showed a value of 4.3. Again 64 and 74 hours after injection, comatose animals showed values of 7.5 and 7.9 respectively while non-comatose toads had values of 5.5 and 4.6 respectively. In Table XXXVII, an experiment is shown where the subsequent rise occurred 45 hours after injection. It is interesting to compare this hyperphosphataemia with the terminal hyperphosphataemia occurring in uraemia (de Wesselow, 1923) and in strychnine convulsions (Harrop and Benedict, 1923).

(d) Mode of action of the pancreas.

A close relationship exists between the pancreas and phosphorus metabolism. Pancreatectomy leads to a rise in plasma inorganic phosphate and insulin injection a fall.

The excessive elimination of phosphates was noted in human diabetics by several clinical workers and this was attributed to the accompanying acidosis (Von Moraczewski, 1897; Van Noorden, 1907 etc.). Allan, Dickson and Markowitz (1924), showed that phlorrhizin produced a marked increase in phosphate excretion and excessive loss of phosphate from the body, after repeated administration of the drug. In diabetes, excessive excretion of phosphate

occurs .....

occurs with the excessive excretion of sugar. It seems probable that there is a high loss of phosphate in severe human diabetes even when proper dietetic treatment is instituted. This may not always be reflected in the blood inorganic phosphate, as inorganic phosphate is required in the blood to maintain the constancy of the H ion concentration of the blood. Conversely when insulin is injected although there is almost complete supression of excretion of phosphate in the urine the blood inorganic phosphate actually falls.

Houssay and Sammartino (1935) have found that after pancreatectomy, the cells of the parathyroids enlarge and become vacuolated. These lesions are unlike those occurring with hypophysectomy. Gerschman and Marenzi (1935b) found a rise of blood inorganic phosphate and a fall of calcium following pancreatectomy. They attribute those changes after pancreatectomy to hypoparathyroidism.
In Xenopus a marked rise of plasma inorganic phosphate occurs within 3 hours after pancreatectomy and this is probably too soon to implicate the parathyroids. The effects of parathyroidectomy are observed within 2 or 3 days after operation in mammals (Dragstedt, 1927). Pancreatectomy produces an effect within 3 hours after operation in a cold-blooded animal. Waggener (1930) showed that parathyroidectomised toads appeared quite normal for one and a half to

two days after operation before the first ill effects of the removal appeared. Injection of insulin produces a fall in blood inorganic phosphate and then a rise above the pre-injection level (Ellsworth and Weinstein, 1933), a fall in urinary phosphate followed by a rise (Sokhey and Allan, 1924). Removal of the pancreas produces the converse, namely a rise in blood inorganic phosphate of the blood (Gerschman and Marenzi, 1935a) and a rise in the amount of phosphate excreted in the urine. (Falta and Whitney, 1908). The results on the plasma inorganic phosphate of Xenopus laevis are in accord with these results. Pancreatectomy produced an immediate rise in inorganic phosphate. Insulin injected into pancreatectomised toads showed a fall towards normal. Insulin injected into normal animals produced an immediate fall and then a rise during hypoglycaemia.

Cori and Cori (1931) emphasized the close similarity of action of insulin and adrenaline. Insulin injection in fasted rats produced a marked increase in hexose phosphate of muscle (Cori and Cori, 1931; Kerr and Bluch, 1932). Adrenaline did exactly the same. Experiments in adrenalectomised rats (Cori and Cori, 1931) showed that there was no change in the hexose phosphate content of muscle with insulin injection even though the blood sugar fell to a very low level. They concluded that the increase in hexose phosphate observed in normal animals after insulin injection

was .....

was due to a reflex discharge of adrenaline elicited by the hypoglycaemia.

The same results were found if glucose was given with insulin to prevent the hypoglycaemia. The diminution of blood and muscle phosphates following insulin injection could be explained by attributing them to a reflex secretion of adrenaline, but not the changes following carbohydrate ingestion. Diminution of blood phosphates occur after a carbohydrate meal and this could not be due to adrenaline.

Pijoan and Quigley (1937) conclude that there are two mechanisms involved. They found that injection of insulin into adrenalectomised animals produced no fall in blood inorganic phosphate whereas glucose administration to adrenalectomised animals produced a fall. Pollack (1933) however, found that intravenous injection of glucose into completely adrenalectomised animals produced a drop in serum inorganic phosphate paralleling the normal but in depancreatised animals there was no fall in inorganic phosphate. Ellsworth and Weinstein (1933) found that insulin produced a more marked depression of serum inorganic phosphate in adrenalectomised dogs than in normal animals. This is contrary to the findings of Pijoan and Quigley (1937).

The bulk of evidence, however, points to insulin as the responsible agent for the fall in blood inorganic

phosphate .....

phosphate following carbohydrate ingestion, the hyperglycaemia produced by the carbohydrate ingestion reflexly stimulating the production of insulin. Dolliger and Hartman (1925) show that sugar ingestion produced no fall in blood inorganic phosphate in pancreatectomised dogs. Markovitz (1926) found no fall in urinary phosphate in depancreatised dogs following glucose administration. Pollack et al (1934) found that intravenous injection of glucose produced no drop in blood inorganic phosphate in depancreatised nor in demuscled animals. If the heart-lung-hind-leg preparation was perfused with glucose there was a marked drop and this was accentuated by insulin. Although the muscles are able to form organic phosphate compounds with glucose this reaction is greatly enhanced by insulin. The muscles appear to be the site of action and not the liver (Dolliger and Hartman, 1925; Pollack et al., 1934).

Experiments on Xenopus laevis permit of the same conclusion (see Pituitary-pancreas-adrenal inter-relationships).

Cori and Cori (1935) have since revised their original hypothesis and attribute the effect of insulin to a dual mechanism.

(a) Reflex secretion of adrenaline which produces a rise in muscle hexose phosphate and thus a fall in blood inorganic phosphate.

(b) a direct effect of insulin itself which leads to the formation of an as yet unknown organic compound in the

tissues .....

tissues from inorganic phosphate.

Before (a) can be accepted, the effect of adrenaline on the plasma inorganic of Xenopus laevis must be determined. This is discussed in the sequel.

The secondary rise of plasma inorganic phosphate following insulin injections is not fully understood and has received little attention. It was noticed in the experiments on Xenopus that the rise occurred particularly when the animals were suffering from severe symptoms of hypoglycaemia. These animals secreted large quantities of mucus and several animals died before the plasma inorganic phosphate could be determined. Bolliger and Hartman (1925) state that during the hypoglycaemia, there is no demand for inorganic phosphate and in consequence the amount rises in the blood. Sugar administration during the hypoglycaemic state lowers the blood inorganic phosphate (Mazzocco and Morera, 1924) and this tends to support the view. Similarly with the administration of glucose the lowest blood inorganic phosphate level comes some time after the peak in blood glucose, the inorganic phosphate is kept low while the glucose is being disposed of and rises to the normal level when the glucose has returned to normal. The decrease of blood inorganic phosphate occurs when the metabolism of glucose is at a peak (Blatherwick, Bell and Hill, 1924).

The probable course of events may be as follows:- While glucose is being oxidised the blood phosphate is

depleted .....

depleted as it is drawn upon for phosphorylating the intermediary products of glucose oxidation and to facilitate glycogenesis in muscle. When hypoglycaemia is present the demand for phosphate is no longer present and consequently a rise in blood inorganic phosphate occurs, the source of which is the bones and soft tissues in all probability. Although adrenaline is reflexly secreted, the effect of insulin overpowers the adrenaline (big doses of insulin are administered sufficient to kill the animal or render it comatose).

The fall in plasma inorganic phosphate following hypophysectomy is probably not due to hypofunction of the pancreas although it may conceivably be due to unopposed action of the pancreatic secretion. To substantiate the argument, experiments showing the effect of insulin on hypophysectomised animals are necessary and these will be discussed in the sequel.

The increase in plasma inorganic phosphate is probably not due to stimulation of the pancreas. It may be due to the cancelling out of insulin action. The secondary fall may be due to reflex secretion of insulin produced as a response to the hyperglycaemia.

SUMMARY .....

SUMMARY.

(1) Ether has no effect on plasma inorganoe phosphate 6 hours after anaesthesia.

(2) Pancreatectomy produces a rise of plasma inorganic phosphate which appears within 3 hours after operation.

(3) Injection of insulin into pancreatectomised animals produces a reduction of plasma inorganic phosphate towards the normal.

(4) Injection of insulin into normal animals produces a fall in plasma inorganic phosphate with a rise above the pre-injection level, when the animals show hypoglycaemic symptoms.

(5) A direct relationship between the pancreas and phosphorus metabolism is suggested. The effect of insulin is a dual one.

(a) reflex secretion of adrenaline

(b) formation of an as yet unknown organic compound, probably in the muscles. The two actions combined produce the fall in plasma inorganic phosphate.

(6) Experiments showing the effect of adrenaline on the plasma inorganic phosphate of Xenopus are required to confirm this hypothesis as to the effect of insulin on Xenopus.

(7) The rise of plasma inorganic phosphate during the hypoglycaemic state is ascribed to the cessation of phosphate withdrawal .....

withdrawal for metabolic purposes and since the call on inorganic phosphate is no longer present the level rises in the blood. Increased excretion of phosphate is not sufficient to prevent a rise of blood inorganic phosphate.

(8) Changes in blood glucose are exactly proportional to changes in plasma inorganic phosphate. On pancreatectomy both rise and with insulin injection both fall.

(9) The fall in plasma inorganic phosphate following hypophysectomy is probably not due to hypoinsulinism as the latter produces a rise. It may, however, be due to unopposed action of insulin. Experiments showing the effect of insulin on hypophysectomised animals are required to substantiate the argument and this is discussed later.

(10) The secondary fall of plasma inorganic phosphate consequent on injection of anterior pituitary extract may possibly be due to reflex stimulation of the pancreas by the hyperglycaemia with the discharge of insulin into the blood stream.

THE EFFECT OF THE ADRENALS ON PLASMA INORGANIC PHOSPHATE

THE ADRENAL GLANDS.

(a) Adrenalectomy.

Attempts at carrying out adrenalectomy were not made because of the anatomy of the organ. The cortex is buried in the ventral half of the kidney and cannot be removed without sacrificing the kidneys as well. The chromaphil cells are scattered in the walls of the renal veins, inferior vena cava, aorta and renal arteries and are impossible to identify apart from removing except microscopically. (Zwavenstein and Schrire, 1932).

(b) Injection of adrenaline

Fresh pond females were used for these experiments, being kept in the laboratory up to a week before use.

About 9 dozen toads were each injected intraperitoneally with 100 μg. adrenaline contained in 0.1 ml. frog Ringer solution. The same number of animals each received an intraperitoneal injection of 0.1 ml. frog Ringer solution and served as controls. After 2 hours several toads injected with adrenaline were found covered with a mucous secretion. This soon disappeared, however, unlike the mucus secreted after insulin injection.

In Tables XLI, XLII and XLIII, the effect of adrenaline on the plasma inorganic phosphate is shown.

Table XLI .....

TABLE XLI.

Plasma inorganic phosphate after adrenaline injection

Pre-injection level 7.6 (3)

Time in hours after injection	Adrenaline 100 µg.	Frog Ringer Solution.
1.5	7.0 (3)	7.9 (3)
3.0	5.5 (4)	8.3 (4)
24.0	6.7 (2)	6.8 (3)

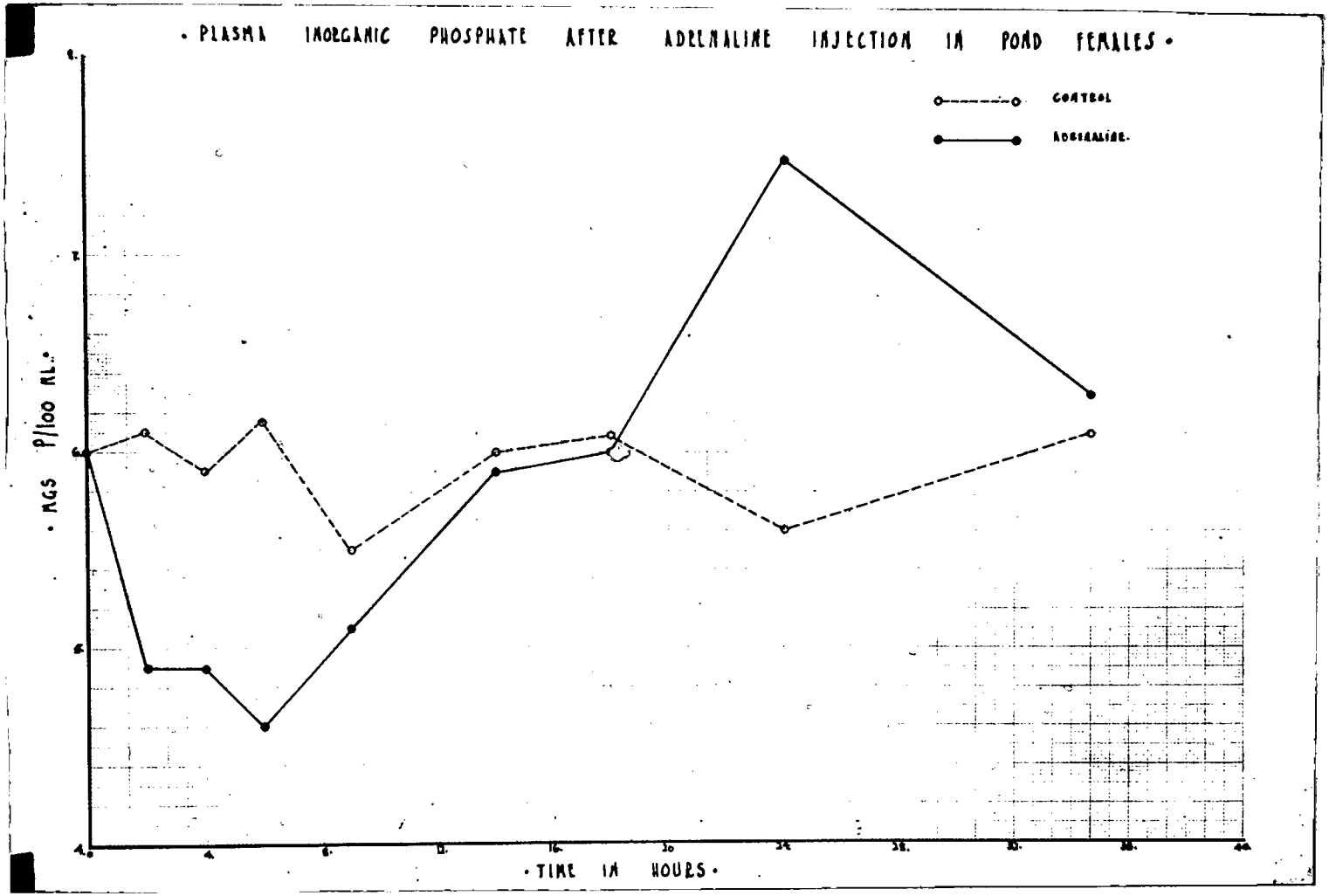
TABLE XLII.

Plasma inorganic phosphate after adrenaline injection

Pre-injection level 6.8 (4)

Time in hours after injection	Adrenaline 100 µg.	Ringer Solution
3	6.1 (4)	7.0 (6)
6	6.2 (5)	7.0 (6)
10	7.3 (4)	6.5 (5)
24	6.6 (4)	6.5 (3)

Table XLIII ...


• PLASMA INORGANIC PHOSPHATE AFTER ADRENALINE INJECTION IN POND FEMALES •
CONTROL
ADRENALINE.
• MGS P/100 ML •
• TIME IN HOURS •

CONTINUED

TABLE XLIII.

Plasma inorganic phosphate after adrenaline injection.

Pre-injection level 6.0±0.23 (5)

Time in hours after injection	Adrenaline 100µg.	Frog Ringer Solution.
2	4.9±0.30 (2)	6.1±0.14 (4)
4	4.9±0.11 (4)	5.9±0.22 (4)
6	4.6±0.20 (2)	6.2±0.25 (3)
9	5.1±0.16 (2)	5.5±0.16 (2)
14	5.9±0.17 (3)	6.0±0.19 (4)
18	6.0±0.21 (4)	6.1±0.25 (2)
24	7.5±0.35 (2)	5.6±0.32 (3)
36	6.3±0.19 (4)	6.1±0.17 (3)

In Tables XLI, XLII and XLIII, each estimation required approximately 2 toads. Detailed figures appear in the appendix:protocol No. 27.

The results shown in Table XLIII are graphically represented below. (Graph XIII).

In Tables XLII and XLIII a subsequent rise in plasma inorganic phosphate is observed.

In Table XLIV below another experiment is shown.

Table XLIV ....

TABLE XLIV.

Plasma inorganic phosphate after adrenaline injection.

Pre-injection level	4.4 ± 0.20	(6)
Adrenaline 12 hours after injection	6.2 ± 0.13	(5)
Control 12 hours after injection	4.1 ± 0.22	(6)

Each estimation required 2 animals. Detailed figures appear in the appendix:protocol No. 27.

DISCUSSION.

(a) Injection of adrenaline.

Tables XLI, XLII, XLIII and XLIV and Graph XIII indicate the effect of adrenaline on the plasma inorganic phosphate.

In Table XLI a maximum fall of 50 per cent (2.6mg.) was observed 3 hours after injection. Unfortunately the controls were very irregular and showed changes of up to 0.8 mg. from the pre-injection level.

In Table XLII the controls remained steady ranging as follows 6.6, 7.0, 7.0, 6.5, 6.5. The adrenaline injected animals showed an initial fall of 13 per cent within 3 hours and a subsequent rise of 12 per cent 10 hours after injection. This experiment was carried out during the

summer .....

summer months.

In Table XLIII controls remained very steady, ranging as follows: (6.0 pre-injection level) 6.1, 5.9, 6.2, 5.5, 6.0, 6.1, 5.6, 6.1. The average value was 6.0 ± 0.06 (25). Adrenaline injected animals showed plasma inorganic phosphate values (6.0) 4.9, 4.9, 4.6, 5.1, 5.9, 6.0, 7.5, 6.3. The fall in plasma inorganic phosphate occurs within 2 hours after injection returns to normal in 14 hours. A maximum fall of 26 per cent was recorded. Subsequently a rise above the normal level is observed, 24 hours after injection, with a return to normal 12 hours later. (see Graph XIII. In Table XLIV the subsequent rise in plasma inorganic phosphate is again observed. These experiments were done early in winter.

In Table XLV below, the significant difference between adrenaline injected and control animals (Table XLIII) is shown.

TABLE XLV.

The significance of figures showing changes in plasma inorganic phosphate following adrenaline injection into normal animals.

Average Control 6.0 ± 0.08 (25)

Time in hours	2	4	6	9	14	18	24	36
Plasma inorganic phosphate	4.9	4.9	4.6	5.1	5.9	6.0	7.5	6.3
Significant difference	50:1	>100:1	75:1	5:1	-	-	50:1	-

(b) Mode of action of adrenaline.

Adrenaline produces an immediate fall of plasma inorganic phosphate within 2 hours of injection in a cold-blooded animal which suggests a direct action on phosphorus metabolism. A subsequent rise in plasma inorganic phosphate was observed 24 hours (Table XLIII, XLIV) 10 hours (Table XLII) after injection. These findings are in keeping with those of Vollmer (1923) and Woringer (1924) and parallel the changes occurring in urinary phosphate excretion, Allan, Dickson and Markowitz (1924). (See introduction)

The effect of adrenaline on the blood inorganic phosphate has been attributed to the action of insulin reflexly secreted as a result of the hyperglycaemia (Barrenscheen et al., 1927). These investigators found that after subcutaneous and intravenous injection of adrenaline the blood inorganic phosphate and the urinary phosphate excretion diminished exactly as it did with insulin. If glucose was given by mouth a reflex secretion of insulin was produced to cope with the new sugar and if adrenaline was injected at the point where the blood sugar was falling i.e. where insulin was acting maximally, there was a rise in blood sugar and inorganic phosphate.

The bulk of evidence, however, is in favour of a direct action of adrenaline on blood inorganic phosphate, in fact the action of insulin was originally attributed to a reflex

secretion .....

secretion of adrenaline (Cori, 1931). Adrenaline produces a temporary rise in muscle hexose phosphate (Cori and Cori, 1931). The rise in muscle hexose phosphate following insulin injection is due to reflex secretion of adrenaline. Muscle hexose phosphate is the precursor of lactic acid and it is well known that adrenaline facilitates the conversion of muscle glycogen to lactic acid, thus increasing the production of hexose phosphate. The temporary accumulation of hexose phosphate throughout the muscles of the body accounts for the fall of blood and urinary phosphate (Cori, 1931).

The subsequent rise in blood and urinary inorganic phosphate has received little attention. It is probably not due to changes in muscle hexose phosphate as will be discussed in the sequel (Adrenaline and pituitary).

Vollmer (1923 a,b,c) claimed that adrenaline caused a rapid diphasic variation in blood constituents. In man, a definite decrease in blood inorganic phosphate and an increase in serum calcium occurs 5 - 15 minutes after injection of adrenaline. This rapid phase is followed by a slow phase in which the inorganic phosphate rises and the calcium falls. The changes in calcium follow the changes in inorganic phosphate. Changes in plasma inorganic phosphate in Xenopus laevis are analogous to those changes

occuring .....

occurring in man after adrenaline injection namely a rapid fall occurring within 2 hours, slowly returning to normal in 14 hours and a slow rise 36 hours after injection. According to Vollmer, the various effects of adrenaline occur in definite sequence. In the first rapid phase, the inorganic phosphate falls and this is followed by a rise in calcium and a drop in potassium. As a result oxidative processes are inhibited (cf. Cori and Cori, (1929) on the effect of adrenaline on carbohydrate oxidation) and this leads to an acidosis. In the second phase, the process is reversed and an alkalosis results. The changes in phosphate and H ion concentration (Gottschalk and Pohle, 1922) are regarded as being of basic importance in determining all the other actions of adrenaline.

The following may be a possible explanation of changes in plasma inorganic phosphate occurring after injection of adrenaline into normal animals. There is an extra demand for phosphate by the muscles for the formation of hexose phosphate and this lowers the blood inorganic phosphate. The total blood phosphate (Labbe et al., 1931) is not changed but there is a rise of organic phosphate concomitant with the fall in inorganic phosphate. The H ion concentration is upset, calcium rises and potassium falls. When the action of adrenaline is abating, for it is rapidly destroyed in the body, a swing back towards normal occurs

and .....

and the inorganic phosphate of blood slowly rises while the calcium falls. The fall in inorganic phosphate may, however, be overcompensated and an oversving occurs producing a temporarily increased plasma inorganic phosphate.

The fall in plasma inorganic phosphate occurring after hypophysectomy is probably not due to deficiency of adrenaline as adrenaline produces a fall in plasma inorganic phosphate. To confirm the suggestion experiments showing the effect of adrenaline on hypophysectomised animals are necessary and these will be discussed in the sequel.

The fall in plasma inorganic phosphate following insulin injection may partly be explained by a reflex secretion of adrenaline evoked by the hypoglycaemia. This will be further discussed in the sequel.

SUMMARY ...

SUMMARY.

(1) Injection of adrenaline into normal animals produces a fall in plasma inorganic phosphate. This is followed by a temporary rise above the pre-injection level.

(2) A direct relationship between adrenaline and phosphorus metabolism is suggested.

(3) The fall in plasma inorganic phosphate is attributed to withdrawal of inorganic phosphate for the formation of hexose phosphate. The accumulation of hexose phosphates in the body is sufficient to account for the fall in blood and urinary inorganic phosphates.

(4) The subsequent rise in plasma inorganic phosphate is attributed to an overswing phenomenon. The initial action of adrenaline is to produce a fall in inorganic phosphate and a rise in calcium follows. The H ion concentration is upset and an acidosis results. This is followed by a slow compensatory phase in which the inorganic phosphate of both blood and urine rise and calcium falls. An overswing occurs leading to a transient rise of plasma inorganic phosphate above the normal level.

(5) The fall in plasma inorganic phosphate following hypophysectomy is probably not due to hyposecretion of adrenaline. Experiments showing the effects of adrenaline

on .....

on hypophysectomised animals are required to confirm the argument and this is discussed later.

(6) Reflex secretion of adrenaline after insulin injection may contribute to the fall of plasma inorganic phosphate occurring after the injection of insulin.

THE INTERRELATIONSHIP OF

THE PITUITARY, THE PANCREAS AND THE ADRENALS.

INTER-RELATION BETWEEN ANTERIOR PITUITARY, PANCREAS AND ADRENALINE.

(a) Anterior pituitary extract on pancreatectomised animals

Animals were pancreatectomised as described and came from the same batch that were used for insulin injections on pancreatectomised animals. The mortality rate was high for reasons already stated.

Fifty two pancreatectomised animals were each injected with 0.4 ml. S.A.P.1B and the same number with 0.25 ml. frog Ringer solution. All injections were given intra-peritoneally. Only 21 animals of each type survived. Injections were given 8 hours after injection for reasons previously stated.

TABLE XLVI ...

TABLE XLVI.

Plasma inorganic phosphate after S.A.P.IB injections into pancreatectomised animals

Pre-operative normal 5.1 (3)

Pancreatectomised (6 hours) Pre-injection level 6.9 (2)

Time in hours after injection	Pancreatectomised toads injected with S.A.P.IB	Pancreatectomised Ringer Solution Control
3	6.5 (2)	6.1 (1)
6	6.1 (2)	6.2 (2)
12	6.0 (3)	6.0 (2)

Each estimation required 4 toads. Detailed figures appear in the appendix:protocol No. 23.

The plasma inorganic phosphate showed evidence of laking and the colorimetric readings could only be approximately estimated. The laking was not due to faulty technique in the collection of the plasma, but was due to laking in vivo.

(b) Injection .....

(b) Injection of insulin into anterior lobe hypophysectomised animals.

Animals were hypophysectomised according to the method of Hogben (1923). The anterior lobe only was removed. The storage and feeding of animals was as described.

The animals were injected with insulin 2 months after the operation. This period was chosen for reasons already stated. The animals were not fed for one week prior to the insulin injections, owing to the fact that food is retained in the gastro-intestinal tract for several days i.e. the experiment was performed in the post-absorptive state. It was observed during the course of this work that more blood was obtained from animals if they were not fed during the preceding week.

About 60 anterior lobe hypophysectomised animals were each injected intraperitoneally with 2 units of crystalline insulin, contained in 0.25 ml. frog Ringer solution and 50 controls with 0.25 ml. frog Ringer solution. The same number of operative controls were similarly injected with insulin and with frog Ringer solution.

Tables XLVIIA, XLVIIB and Graph XIV show the effect of insulin on hypophysectomised and operative control animals.

Table XLVIIA .....

TABLE XLVIIA.

Plasma inorganic phosphate after injection of insulin (2 units) into anterior lobe hypophysectomised animals.

Pre-operative normal	5.2 ± 0.16 (5)
Hypophysectomised animals	4.5 ± 0.11 (4)

Time in hours after injection	Insulin 2 units	Ringer Solution Control.
4	3.2 ± 0.16 (4)	3.9 ± 0.09 (4)
8	3.6 ± 0.15 (4)	4.3 ± 0.13 (4)
12	3.9 ± 0.11 (5)	4.3 ± 0.23 (3)
19.5	3.8 ± 0.14 (6)	4.5 ± 0.12 (3)
25	3.9 ± 0.21 (6)	4.8 ± 0.19 (4)
37.5	4.0 ± 0.13 (5)	(4.2 ± 0.20 (2))
50	4.2 ± 0.10 (4)	4.7 ± 0.10 (2)

TABLE XLVIIB ..

GRAPH "IV".

PLASMA INORGANIC PHOSPHATE AFTER INSULIN INJECTION IN HYPOPHYSECTOMISED ANIMALS.
HYPOPHYSECTOMY INSULIN.
HYPOPHYSECTOMY RINGER SOLUTION.
OPERATIVE CONTROL INSULIN.
OPERATIVE CONTROL RINGER SOLN.
MG. P/100 ML.
TIME IN HOURS.

TABLE XLVIIB.

Plasma inorganic phosphate after injection of insulin (2 units) into operative control animals.

Pre-operative normal	5.2 ± 0.16 (5)
Operative Control animals	5.3 ± 0.09 (5)

Time in hours after injection	Insulin 2 units	Ringer Solution Control
4	3.7 ± 0.11 (4)	4.8 ± 0.15 (3)
8	4.0 ± 0.05 (5)	5.3 ± 0.26 (3)
12	4.0 ± 0.07 (5)	5.6 ± 0.34 (3)
19.5	3.4 ± 0.12 (7)	5.3 ± 0.25 (2)
26	4.2 ± 0.08 (7)	5.6 ± 0.21 (3)
37.5	4.3 ± 0.17 (6)	5.9 ± 0.29 (3)
50	5.0 ± 0.18 (5)	5.3 ± 0.27 (4)

Each estimation required 2 animals. Detailed figures appear in the appendix:protocol No. 23.

Only one hypophysectomised animal secreted mucus after 50 hours.

The above results are graphically represented below. (Graph XLV)

In .....

In Table XLVIII below the effect of insulin (1 unit in 0.5 ml. solution is shown.

TABLE XLVIII.

Plasma inorganic phosphate in hypophysectomised animals 2 hours after injection with insulin (1 unit).

Pre-injection level	4.9	(3)
6 hours after injection	5.2	(4)

Each estimation required 2 animals. Detailed figures appear in the appendix:protocol No. 28.

(c) Injection of adrenaline into anterior lobe hypophysectomised animals.

Animals from the same batch as those used in the experiments with insulin were used. Adrenaline was injected a few days after the insulin experiments had been completed so that food was withheld from these animals for 10 days. In the insulin experiments hypophysectomised and operative controls injected with frog Ringer solution showed no change. Since the adrenaline experiments were carried our immediately after the insulin experiments, it was thought unnecessary to inject new controls.

About .....

About 100 hypophysectomised animals were each injected, intraperitoneally, with 100 µg. adrenaline in 0.25 ml. frog Ringer solution. The same number of operative controls were similarly injected with adrenaline. A transient secretion of mucus was observed in both types of animals after 2 hours just as in normal animals.

In Tables XLIX A, XLIX B and L and Graph XV, the effect of adrenaline on hypophysectomised toads is shown.

TABLE XLIX A.

Plasma inorganic phosphate after injection of adrenaline into anterior lobe hypophysectomised animals.

Pre-operative normal 5.2±0.16 (5)

Hypophysectomised animals 4.5±0.07 (6)

Time in hours after injection	Adrenaline 100µg.
2	4.9 ± 0.13 (4)
3	4.9 ± 0.12 (3)
4	4.7 ± 0.09 (3)
5	4.7 ± 0.20 (2)
12	4.6 ± 0.17 (3)
19	5.6 ± 0.15 (3)
25	5.4 ± 0.07 (3)
36	6.1 ± 0.10 (2)

Table XLIX B .....

GRAPH XV.

• PLASMA INORGANIC PHOSPHATE AFTER ADRENALINE INJECTION IN HYPOPHYSECTOMISED ANIMALS.
HYPOPHYSECTOMY.
OPERATIVE CONTROL.
• MG. P/100 ML. •
• TIME IN HOURS.

TABLE XLIXD.

Plasma inorganic phosphate after injection of adrenaline into operative control animals

Pre-operative normal	5.2 ± 0.16 (5)
Operative control animals	5.3 ± 0.09 (5)

Time in hours after injection	Adrenaline 100µg.
2	5.5 ± 0.20 (3)
3	4.7 ± 0.16 (2)
4	4.7 ± 0.07 (2)
5	5.0 ± 0.25 (2)
6	4.7 ± 0.12 (3)
12	5.5 ± 0.16 (2)
19	6.2 ± 0.09 (4)
25	6.8 ± 0.15 (3)
36	6.6 ± 0.10 (2)

Each estimation required approximately 4 animals. Detailed figures appear in the appendix:protocol No. 29.

Table L .....

TABLE I.

Hypophysectomy Pre-injection level 4.5 ± 0.07 (6)

Operative Control Pre-injection level 5.3 ± 0.09 (5)

Time in hours after injection	adrenaline injected hypophysectomised	adrenaline injected Operative controls
3	4.6 ± 0.30 (2)	4.0 ± 0.07 (2)

Each estimation required approximately 4 toads.

Detailed figures appear in the appendix protocol No. 29.

DISCUSSION ....

DISCUSSION.

(a) Anterior pituitary extract in pancreatectomised animals.

In a previous discussion it was suggested that the secondary fall occurring with anterior pituitary injection may be due to a reflex secretion of insulin. This experiment was undertaken to test out the hypothesis for if the hypothesis were valid no secondary fall would be expected in pancreatectomised animals. Unfortunately, however, the results of this experiment are wholly unreliable because of the heavy mortality amongst operated animals. This mortality was as great amongst control as amongst pancreatectomised animals and must be ascribed to faulty post-operative technique. The issue therefore remains in doubt.

(b) Insulin in anterior lobe hypophysectomised animals.

Hypophysectomy produced a fall in plasma inorganic phosphate (15 per cent). The hypophysectomised animals injected with frog Ringer solution as controls showed little change the values ranging as follows:-
4.5, 3.9, 4.3, 4.3, 4.5, 4.6, 4.2, 4.7. The average value is 4.4 ± 0.09 (22). Hypophysectomised animals injected with insulin showed the following values:-
(4.5), 3.2, 3.6, 3.9, 3.8, 3.9, 4.0, 4.2.
Insulin therefore produces a fall in plasma inorganic phosphate in hypophysectomised animals.

Operative ...

Operative controls injected with frog Ringer solution as controls showed little change the values ranging as follows:- 5.3 (4.8), 5.3, 5.6, 5.3, 5.6, 5.9, 5.6.
The average value is 5.4 ± 0.11 (21). Operative controls injected with insulin showed the following values:-

(5.3), 3.7, 4.0, 4.0, 3.3, 4.2, 4.3, 5.0.

Insulin thus produces a fall in plasma inorganic phosphate in operative control animals and this fall is much more marked than in hypophysectomised animals. This fact is well brought out in Graph XIV and Table LI below.

TABLE LI

Percentage difference in plasma inorganic phosphate in hypophysectomised and operative controls following insulin injection.

Time in hours after injection	4	8	12	19	25	37	50
Hypophysectomy	-18	-16	-9	-16	-19	-5	-11
Operative Control	-23	-25	-29	-38	-26	-27	-6

The fall in plasma inorganic phosphate following insulin injection is significantly greater in operative controls than in hypophysectomised animals.

(c) Adrenaline ....

(c) Adrenaline in anterior lobe hypophysectomised animals.

Hypophysectomised animals injected with adrenaline showed no preliminary fall in plasma inorganic phosphate (Tables XLIX A and L and Graph XV). The following plasma inorgaic phosphate values were observed:-
(4.5 pre-injection value),4.9,4.9,4.7,4.7,4.6,5.6,5.4,6.1.
The subsequent rise in plasma inorganic phosphate, however, occurred. Operative controls injected with adrenaline showed the following values:-
(5.3 pre-injection value),5.5,4.7,4.7,5.0,4.7,5.5,6.2,6.7,6.6.
In operative control animals adrenaline produced a fall in plasma inorganic phosphate but this fall is less pronounced than in animals fresh from the pond. The subsequent hyperphosphataemia occurred as in normal animals.

In Table L a fall of 25 per cent in operative controls but no change in hypophysectomised animals after adrenaline injection was observed.

Adrenaline thus produces a fall in plasma inorganic phosphate in operative control but not in hypophysectomised animals. The secondary hyperphosphataemia occurs in both types of animals. These observations are well brought out in Graph XV and in Table LII below.

Table LII...

TABLE LII.

Percentage changes in plasma inorganic phosphate in hypophysectomised and in operative controls following adrenaline injection.

Time in hours after injection	2	3	4	5	6	12	19	25	36
Hypophysectomy	+9	+4	+4	+4		+2	+24	+20	+35
Operative Control	+2	-13	-13	-8	-13	+2	+15	+24	+22

Mode of action of insulin and adrenaline in hypophysectomised animals.

It is well established that the removal of the anterior lobe of the pituitary results in an increased sensitivity to insulin as shown by the effect on blood glucose. This subject has been recently reviewed by Russell (1938) and Young (1939).

In the absence of the thyroid the hypersensitivity to insulin also appears, but is developed more slowly and is usually less pronounced after thyroidectomy than after hypophysectomy (Duchenau, 1924; Houssay and Busso, 1924; Britton and Myers, 1925; Burn and Marks, 1925; and Cope and Marks, 1934). The effect of hypophysectomy on the response to insulin is thus probably not due to the thyroid (Russell, 1938).

The .....

The removal of the adrenal medulla also leads to an increased sensitivity to insulin and this is supposed to be due to lack of adrenaline which is reflexly elicited by the hypoglycaemia (Levis et al., 1925; Britton et al., 1926; Lucke et al., 1933; Barnes et al., 1934). Zucker and Berg (1937) consider that lack of adrenaline does not affect the recovery from insulin hypoglycaemia in normal dogs.

The adrenal medullae appear normal after hypophysectomy, adrenaline is present but the animal is supposed to be resistant to its effects. Bachman and Toby (1936) suggest that the impairment of hyperglycaemic responses to adrenaline in hypophysectomised animals may be due to the relative fixation of muscle glycogen. Chaikoff et al., (1935b) came to a similar conclusion. Corkhill, Marks and White (1933) commented on the extraordinary sensitivity to insulin and showed that lack of carbohydrate was not responsible for the increased sensitivity. Injections of adrenaline which usually relieve insulin hypoglycaemia have little or no effect and they suggest that the glycogen reserves are abnormally resistant to the action of adrenaline. Cope and Marks (1934) using denervated pupil and isolated rabbit intestine preparations have shown that the adrenal glands respond normally to insulin hypoglycaemia by liberating adrenaline into the blood. The adrenaline, however, failed to raise the blood sugar to the

normal .....

normal level in spite of the presence of ample reserves of liver glycogen.

Russell and Cori (1937) pointed out that in most of these experiments the adrenaline had been administered subcutaneously. They found a diminished formation of muscle hexose phosphate in hypophysectomised rats, when the adrenaline was administered subcutaneously. Intravenous injections, however, gave the same results as either subcutaneous or intravenous injection into the normal intact rat. They conclude from this experiment that the diminished effect of adrenaline in hypophysectomised animals is not due to an increased resistance of muscle glycogen to the mobilising action of adrenaline. They suggest that it is due to the diminished absorption of adrenaline from the subcutaneous site of injection.

On the other hand, it has been noted by Cope and Marks (1934) and by Houssay and di Benedetto (1933) that injection of anterior pituitary extracts besides diminishing the sensitivity to insulin exagerated the hyperglycaemic effects of subcutaneously injected adrenaline. Young (1939b), however, has shown that in rabbits treated with glycotrophic substance the hyperglycaemia action of subcutaneously injected adrenaline is substantially increased but not if the adrenaline is infused intravenously.

The effect of insulin and of adrenaline on the blood

inorganic .....

inorganic phosphate has not been extensively investigated. Insulin, and adrenaline in normal animals produce a fall in blood inorganic phosphate. Chaikoff et al., (1935a) found that the effect of insulin on the blood inorganic phosphate was much greater in hypophysectomised than in normal dogs. Chaikoff et al., (1935b) obtained a diminished fall with subcutaneously injected adrenaline, however. They suggest that in addition to absence of the anterior lobe, cerebral manipulations contributed to the results.

In Xenopus laevis the response to insulin is very much less in hypophysectomised than in operative controls. According to Cori's hypothesis, insulin has a dual mode of action:-

(a) reflex secretion of adrenaline producing a fall in blood inorganic phosphate,

(b) a direct effect of insulin itself.

The muscle glycogen may be abnormally resistant to the action of adrenaline in hypophysectomised animals (Bachman and Toby, 1936). Corbhill and Marks (1933) showed that adrenaline is secreted during insulin action, but it has no effect on the blood sugar. Chaikoff et al (1935b) found that subcutaneous adrenaline had a diminished effect on the inorganic phosphate of hypophysectomised dogs. Experiments on Xenopus show that in contrast to the effect on normal animals, injections of adrenaline have no effect

on .....

on the plasma inorganic phosphate of hypophysectomised animals. Thus the adrenaline reflexly secreted as a result of insulin hypoglycaemia has no effect on the blood inorganic phosphate in hypophysectomised animals. The initial fall observed must, therefore, be ascribed solely to the effect of insulin. It is to be expected that this would be less than in normal animals in which both mechanisms contributed to the fall. If Table LI is consulted, it will be observed that during the first 8 hours the difference between operative controls and hypophysectomised animals is not as marked as during the 12 - 39 hours when the hypoglycaemia is at its height.

The fall in plasma inorganic phosphate following hypophysectomy cannot be attributed to pancreatic deficiency because - (1) pancreatic deficiency leads to a rise in plasma inorganic phosphate,

(2) injection of insulin into hypophysectomised animals does not restore the inorganic phosphate level, but produces a further depression.

Chaikoff et al (1935b) found that the response of the sugar lactic acid and inorganic phosphate of the blood to the subcutaneous injection of adrenaline was diminished in hypophysectomised dogs. Since the blood lactic acid curve runs parallel with the rise and fall of hexose phosphate content of muscle during adrenaline action (Cori and Cori, 1931) .....

1931) they concluded that there is less hexose phosphate formed in hypophysectomised animals. The fall in inorganic phosphate, which depends on the amount of hexose phosphate formed is, therefore, less marked.

In Xenopus, intraperitoneal injection of adrenaline produces a fall of plasma inorganic phosphate in normal animals, in operative controls, but not in hypophysectomised animals. Absorption from the peritoneal cavity occurs through a thin layer of peritoneal cells and capillary endothelium. Either the muscle glycogen of this species is resistant to the mobilising action of adrenaline or hypophysectomy has some effect on the absorption of adrenaline from the peritoneal cavity, analogous to the effect on the subcutaneous absorption in the rat. If the latter hypothesis is adopted, the diminished response of hypophysectomised animals to insulin remains unexplained.

The fall in plasma inorganic phosphate following hypophysectomy cannot be ascribed to adrenaline deficiency because - (1) adrenaline produces a fall in plasma inorganic phosphate,

(2) injection of adrenaline into hypophysectomised animals does not restore the inorganic phosphate level of hypophysectomised animals.

Insulin produces its effect on plasma inorganic phosphate by a direct action of its own and adrenaline is only

a .....

a contributory factor.

The subsequent rise in plasma inorganic phosphate following adrenaline injection is apparently independent of the anterior pituitary as the rise also occurs in hypophysectomised animals. This hyperphosphatemia, therefore, is presumably not due to changes in muscle hexose phosphate. It may possibly be due to the effect of adrenaline on the blood inorganic constituents (Vollmer 1923). In the hypophysectomised animal, there is no extra demand for inorganic phosphate by the muscles. The changes brought about by adrenaline on the blood constituents leading to an inhibition of the oxidative processes may occur, but the acidosis is masked, the phosphate demands being easily met by the tissues. The subsequent rise in plasma inorganic phosphate may be due to the slow compensatory processes adjusting the relationships of the various plasma constituents and the acidosis, excess phosphate being now excreted.

SUMMARY .....

SUMMARY.

(1) Injection of insulin into hypophysectomised animals produces a decreased diminution of plasma inorganic phosphate as compared with operative controls. It is suggested that the cause is the inability of adrenaline, reflexly secreted, to assist in lowering the plasma inorganic phosphate. This may possibly be due to abnormal stability of muscle glycogen and consequently of the organic phosphorus compounds.

(2) Insulin depresses plasma inorganic phosphate partly by a direct action on phosphorus metabolism. The complete effect of insulin in normal animals is a dual one, owing to (a) a reflex secretion of adrenaline,

(b) formation of an as yet unknown organic compound. The two actions combined produce the whole fall in plasma inorganic phosphate. In hypophysectomised animals, the action of adrenaline is cancelled out, so that only the formation of organic compounds takes place and as a result, the demand on inorganic phosphate is less than in normal animals where both mechanisms are at work.

(3) The fall in p asma inorganic phosphate following hypophysectomy cannot be due to hypofunction of the pancreas because - (a) Pancreatic deficiency leads to a rise in plasma inorganic phosphate,

(b) Injection of insulin into hypophysectomised animals .....

animals does not restore the inorganic phosphate level, but produces a further depression.

(4) The anterior pituitary is necessary for the complete action of adrenaline on plasma inorganic phosphate. Absence of the anterior pituitary leads to no initial fall of plasma inorganic phosphate after adrenaline injection.

(5) This is attributed to an abnormal stability of muscle glycogen to the mobilizing action of adrenaline. Small changes in hexose phosphate occur and consequently little extra demand for inorganic takes place.

(6) The secondary rise in plasma inorganic phosphate after adrenaline injection is presumably not due to changes in muscle hexose phosphate but may possibly be due to slow compensatory processes adjusting the relationships of the various plasma constituents.

The anterior pituitary is not required for this.

(7) The fall in plasma inorganic phosphate following hypophysectomy cannot be due to hypofunction of the adrenal medulla because - (a) adrenaline produces a fall in plasma inorganic phosphate,

(b) injection of adrenaline into hypophysectomised animals does not restore the inorganic phosphate level.

THE INTERRELATIONSHIP BETWEEN THE PITUITARY AND THE GONADS.

INTER-RELATIONSHIP BETWEEN ANTERIOR PITUITARY AND GONADS.

(a) Anterior pituitary extract in ovariectomised animals.

The animals were ovariectomised according to the method of Shapiro (1933b). The operation was performed during July i.e. at the height of the breeding season. Storage and feeding was carried out as previously described. Food was withdrawn for one week prior to injection for reasons discussed on page 204.

Animals were injected 2 months after operation. 41 ovariectomised animals were each injected intraperitoneally with 0.4 ml. anterior pituitary extract (S.A.P.2B) and 29 with the same volume of brain extract. The same number of operative controls was injected. Throughout the experiment the animals were kept in a room artificially warmed to 19.5°C. An ovulation count was made on operative control animals injected with S.A.P.2B according to the method of Zuckerstein (1937). Post-mortem examinations were made on every animal after it had been bled. In every gonadectomised animal complete removal had been effected. In the operative control animals, the ovaries were in excellent condition.

In Tables LIIIA and LIIIB the effects of anterior pituitary extract on gonadectomised and operative control animals is shown. These results are graphically represented below. (Graph XVI).

TABLE LIIIA ....

GRAPH XVI.

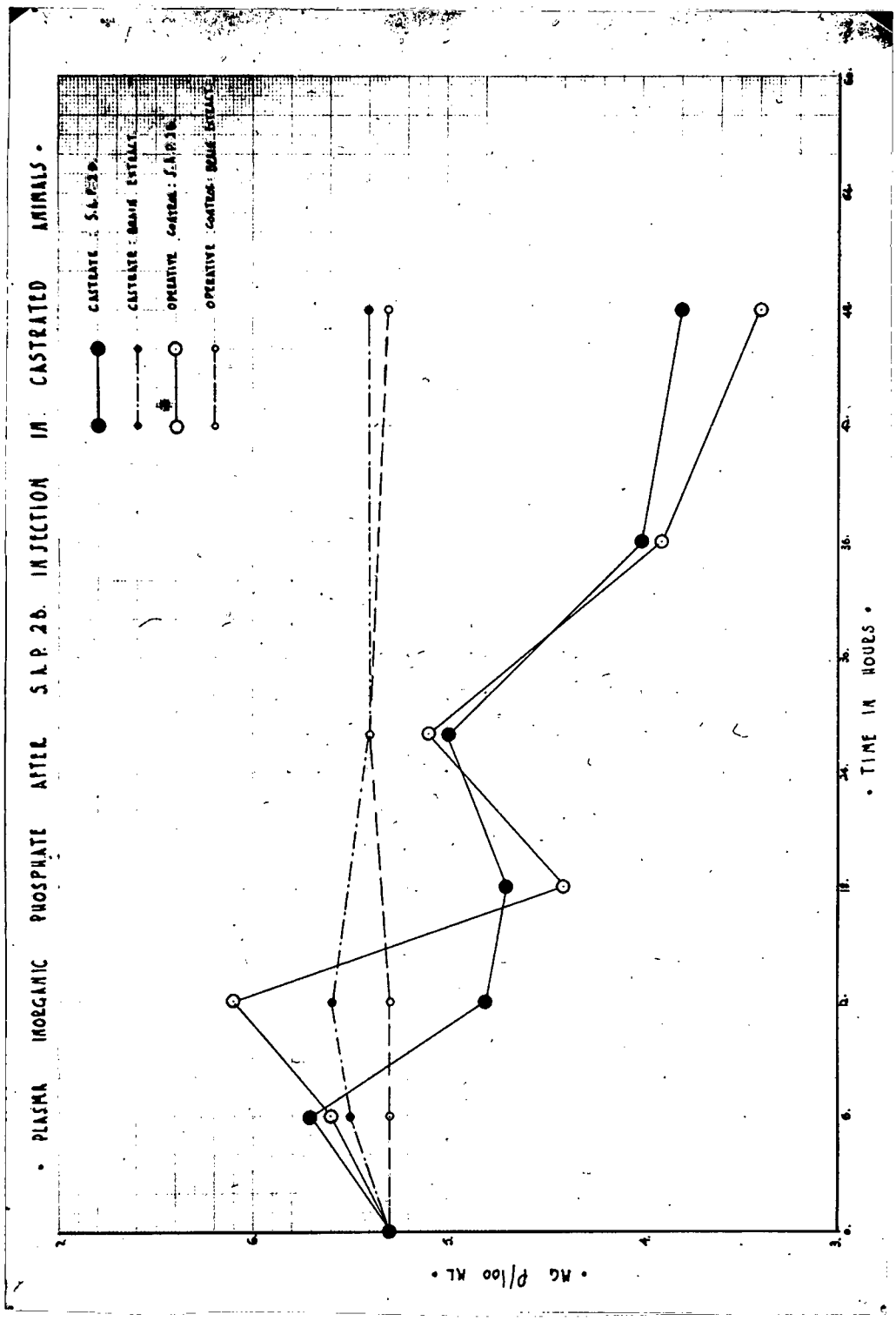

• PLASMA INORGANIC PHOSPHATE AFTER S.A.P. 2b. INJECTION IN CASTRATED ANIMALS •
CASTRATE : S.A.P.2b.
CASTRATE : BOVINE ESTRADIOL
OPERATIVE CONTROL : S.A.P.2b.
OPERATIVE CONTROL : BOVINE ESTRADIOL
• MG P/100 ML •
• TIME IN HOURS •

TABLE LIIIA

Plasma inorganic phosphate after injections of S.A.P.2B. into ovariectomised animals.

Pre-operative normal	4.7 ± 0.08 (11)
Two months after ovariectomy	5.3 ± 0.15 (10)

Time in hours after injection	S.A.P.2B	Brain Extract Control.
6	5.7±0.17 (7)	5.5±0.17 (6)
12	4.8±0.32 (6)	5.6±0.08 (5)
18	4.7±0.14 (7)	
26	5.0±0.15 (8)	5.4±0.20 (7)
36	4.0±0.10 (9)	
48	3.8±0.37 (5)	5.4±0.20 (6)

TABLE LIIIB .....

TABLE 111b.

Plasma inorganic phosphate after injections of S.A.P.2B into operative controls.

Pre-operative normal 4.7 ± 0.08 (11)

Two months operative control 5.3 ± 0.22 (8)

Time in hours after injection	S.A.P.2B	Brain Extract Control	Ovulation Index
6	5.6±0.07 (5)	5.3±0.23 (5)	3/3 1
12	6.1±0.16 (6)	5.3±0.16 (4)	8/4 2
18	4.6±0.15 (6)		29/7 4
26	5.1±0.39 (3)	5.4±0.15 (6)	17/5 3
36	3.9±0.11 (4)		14/5 3
48	3.3±0.16 (4)	5.3±0.15 (5)	

Approximately 1 toad was used for each estimation. Detailed figures appear in the appendix:protocol No. 30.

(b) Oestradiol benzoate and progesterone in anterior lobe hypophysectomised animals.

Animals were hypophysectomised according to the method of Hogben (1923). The anterior lobe only was removed. The storage and feeding of animals was as described on page 67

The animals were injected with oestradiol benzoate and progesterone 2 months after the operation. This period was chosen for reasons given on page 113. The animals were not fed for one week prior to the injections.

About 43 hypophysectomised animals were each injected with 500 µg. progesterone, contained in 0.1 ml. oil; 42 with 250 µg. oestradiol benzoate contained in 0.1 ml. oil and 28 with 0.1 ml. nut oil.
The same number of operative controls was injected.
All injections were given intraperitoneally.
Ovulation counts were made on the progesterone injected animals.
Animals were kept in a room artificiallyheated to 18°C throughout the experiment.

Tables LIVA and LIVB and Graphs XVII and XVIII show the effects of progesterone and oestradiol benzoate on hypophysectomised and operative control animals.

TABLE LIVA ....

GRAPH XVII.

• PLASMA INORGANIC PHOSPHATE AFTER PROGESTERONE INJECTION IN HYPOPHYSECTOMISED ANIMALS •
HYPOPHYSECTOMY + PROGESTERONE.
HYPOPHYSECTOMY + NUT OIL.
OPERATIVE CONTROL + PROGESTERONE
OPERATIVE CONTROL + NUT OIL.
• MG. P/100 ML. •
• TIME IN HOURS •

TABLE LIV A.

Plasma inorganic phosphate after injection of progesterone (500 µg.) and oestradiol benzoate (250 µg.) into anterior lobe hypophysectomised animals.

Pre-operative normal	4.9 ± 0.24 (5)
Two month hypophysectomy	4.5 ± 0.06 (3)

Time in hours after injection	Progesterone 500µg.	Oestradiol B 250µg.	Oil Control	Ovulation Index
12	4.1±0.09 (4)	3.7±0.15 (6)	4.8±0.14 (4)	0
24	3.0±0.04 (3)	3.5±0.23 (5)	4.5±0.12 (3)	3/3 1
36	3.3±0.32 (4)	4.0±0.12 (5)	4.4±0.14 (4)	0
48	3.8±0.20 (4)	3.3±0.11 (4)	4.5±0.07 (2)	8/2 4
72	4.1±0.14 (6)	3.7±0.20 (4)		4/2 2
96	3.5±0.09 (5)	3.8±0.70 (2)		8/2 4

TABLE LIV B .....

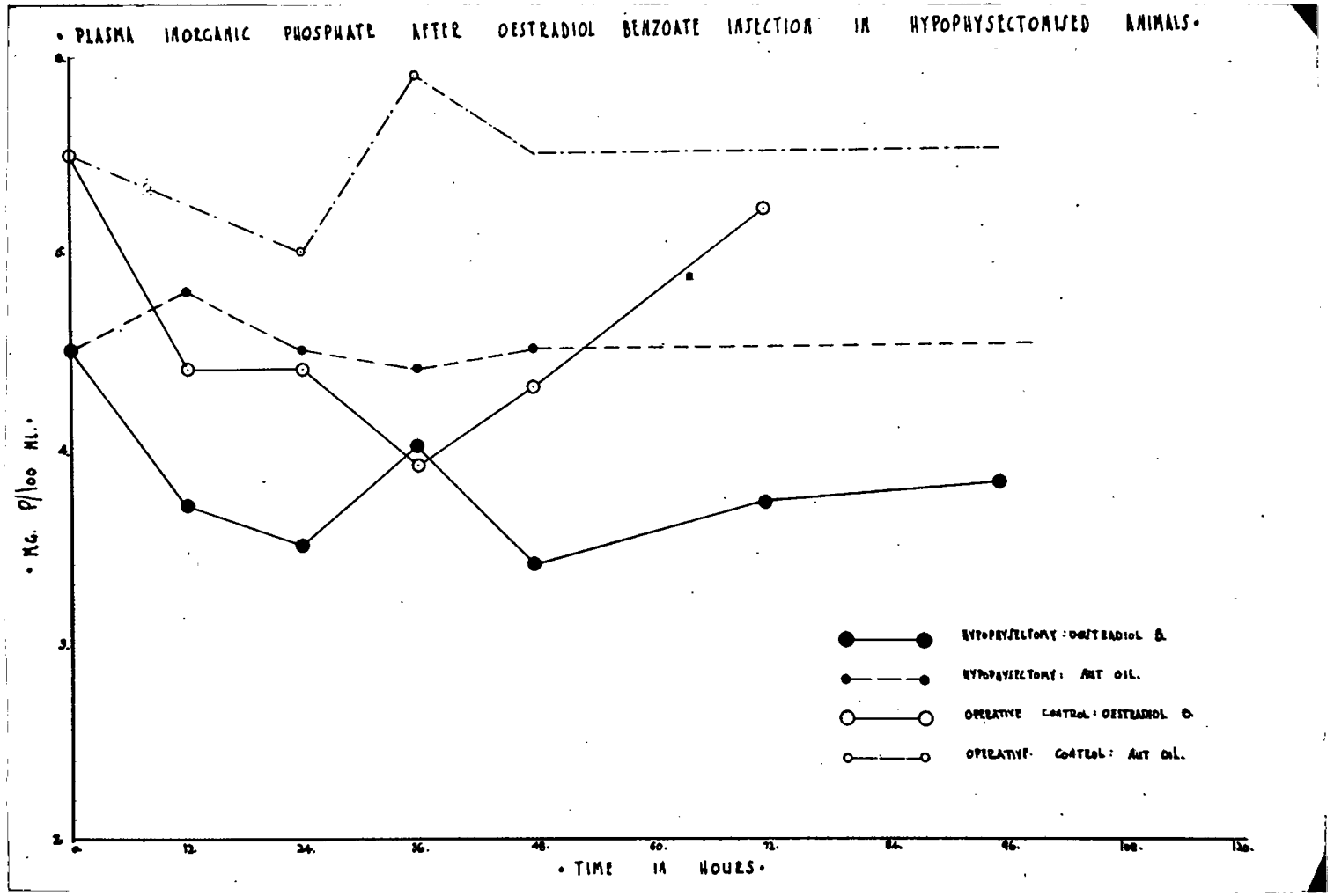

• PLASMA INORGANIC PHOSPHATE AFTER OESTRADIOL BENZOATE INJECTION IN HYPOPHYSECTOMISED ANIMALS.
• MG. P/100 ML.
6.
5.
4.
3.
2.
0.
12.
24.
36.
48.
60.
72.
84.
96.
108.
120.
• TIME IN HOURS.
HYPOPHYSECTOMY : OESTRADIOL B.
HYPOPHYSECTOMY : ART OIL.
OPERATIVE CONTROL : OESTRADIOL B.
OPERATIVE CONTROL : ART OIL.

TABLE LIV B.

Plasma inorganic phosphate after injection of progesterone (500 µg.) and oestradiol benzoate (250 µg.) into operative control animals.

Pre-operative normal	4.9 ± 0.24 (5)
Two month operative control	5.5 ± 0.09 (4)

Time in hours after injection	Progesterone 500 µg.	Oestradiol 250 µg.	Oil Control	Ovulation Index
12	5.0±0.09 (4)	4.4±0.11 (4)	※	1/1 1
24	4.1±0.32 (4)	3.9±0.35 (4)	5.0±0.35 (3)	5/2 2
36	3.7±0.21 (4)	3.9±0.10 (4)	5.9±0.23 (3)	0
48	4.4±0.09 (5)	4.3±0.14 (4)		3/6 6
72	4.1±0.12 (4)	5.2±0.45 (3)		20/5 4
96	4.1±0.30 (4)			20/5 4

※ Animals escaped.

Each estimation required approximately 2 animals. Detailed figures appear in the appendix:protocol No. 31.

DISCUSSION...

DISCUSSION.

(a) Anterior pituitary extract in ovariectomised animals

Brain extract had no effect on the plasma inorganic phosphate, either in the ovariectomised, or in the operative control animals. In ovariectomised, the following figures were recorded:-

(5.3 pre-injection level) 5.5, 5.6, 5.4, 5.4.

In operative controls the following figures:-

(5.3 pre-injection level) 5.3, 5.3, 5.4, 5.3.

Ovariectomy had no effect on the plasma inorganic phosphate after 2 months.

Ovariectomised animals injected with S.A.P.2B showed the following changes:- (5.3), 5.7, 4.8, 4.7, 5.0, 4.0, 3.8.

while operative controls showed the following:-

(5.3), 5.6, 6.1, 4.6, 5.1, 3.9, 3.4.

It is obvious that the only difference between the two groups of animals occurred 12 hours after injection. This is well seen in Graph XVI and Table LV below.

TABLE LV .....

TABLE LV.

Percentage change in plasma inorganic phosphate in castrated and in operative controls following injection of S.A.P.^{2}S.

Time in hours after injection	6	12	18	26	36	48
Ovariectomised	+4	-13	-13	-7	-26	-30
Operative Controls	+6	+15	-15	-6	-26	-32

Anterior pituitary extract injection in operative control animals produces the same effect as in normal animals, namely, a primary rise in plasma inorganic phosphate followed by a secondary fall below the pre-injection level. In castrated animals, however, the primary rise did not occur, but the secondary fall was as great as in operative controls. In a previous discussion it was considered that the injection of anterior pituitary extract produced a primary rise in plasma inorganic phosphate by virtue of a direct action on phosphorus metabolism just as hypophysectomy led to a fall in plasma inorganic phosphate. It was suggested that the secondary fall might be produced by stimulation of the gonads, especially as oviposition occurred just about at the time when the fall became manifest. However, the fall in plasma inorganic phosphate is

not .....

not due to ovarian stimulation as it occurs whether the ovary is present or not.

It appears from the above experiment that the primary rise in plasma inorganic phosphate requires the presence of the ovary. Too much emphasis must not be put on this, however, as this conclusion depends on only one figure, namely 4.6 appearing 12 hours after injection. It is possible that this figure may be inaccurate and the experiment requires confirmation. It will be observed on referring back to experiments on injection of anterior pituitary extract into normal and anterior lobe hypophysectomised animals, that a rise occurred within 6 hours of injection regularly. Moreover, the secondary fall in plasma inorganic phosphate never occurred as soon as 12 hours after injection but usually appeared within 24 hours after injection. Also injection of anterior pituitary extract in hypophysectomised animals produced a rise in plasma inorganic phosphate as soon as 6 hours later, although the ovaries of hypophysectomised animals tend to atrophy and degenerate.

(b) Injection of progesterone and oestradiol in anterior lobe hypophysectomised animals.

Hypophysectomised and operative controls injected with oil showed little change. In the hypophysectomised animals, the following figures were recorded:-

(4.5 .....

(4.5 pre-injection level), 4.6, 4.5, 4.4, 4.5.

In the operative controls the following figures

(5.5) 5.0, 5.9.

Hypophysectomy again produced a fall in plasma inorganic phosphate (18 per cent).

Hypophysectomised animals injected with ovarian hormones showed the following changes:-

(4.5), 4.1, 3.0, 3.3, 3.6, 4.1, 3.5. Progesterone

(4.5), 3.7, 3.5, 4.0, 3.4, 3.7, 3.6. Oestradiol benzoate.

Operative controls injected with progesterone and oestradiol benzoate showed the following:-

(5.5), 5.0, 4.1, 3.7, 4.4, 4.1, 4.4. Progesterone

(5.5), 4.4, 4.4, 3.9, 4.3, 5.2, Oestradiol benzoate.

It will be seen that there is little difference in the response of hypophysectomised or operative control animals to oestradiol benzoate or progesterone. This is well seen in Graph XVII and Table LVI below.

TABLE LVI .....

TABLE LVI.

Percentage changes in plasma inorganic phosphate in hypophysectomised and operative controls following injections of progesterone and oestradiol benzoate.

Time in hours after injection	12	24	36	48	72	96
Progesterone hypophysectomised	-15	-33	-25	-16	-9	-22
Operative Control	-9	-18	-37	-20	-25	-25
Oestradiol Hypophysectomised	-23	-22	-9	-24	-18	-16
Operative Control	-20	-12	-33	-21	-5	

Injection of progesterone and of oestradiol benzoate has the same effect on operative controls, hypophysectomised and normal animals, namely a prolonged fall of plasma inorganic phosphate.

It is, therefore, obvious that these mammalian ovarian extracts act by directly affecting phosphorus metabolism without the intervention of the anterior pituitary. The fall in plasma inorganic phosphate consequent on injection of progesterone is thus not due to inhibition of the

anterior .....

anterior lobe of the pituitary.

The fall of plasma inorganic phosphate following progesterone injection is not due to amphibian hormone liberated during the process of ovulation as it occurs in hypophysectomised animals where the ovulation was comparatively meagre. Moreover, it occurs in oestradiol injected animals in which oestradiol has no effect on the labia or accessory sexual organs. It is important to note that progesterone is gonadotrophic in female Xenopus (unlike in mammals where its action is not on the ovary but on the secondary sex characters). In the normal female Xenopus, progesterone produces ovulation and congestion of the cloacal labia. This does not occur in the gonadectomised animal.

SUMMARY.....

SUMMARY.

(1) Injection of anterior pituitary extract into ovariectomised animals produces a fall in plasma inorganic phosphate similar to that occurring in operative control animals. The secondary fall in plasma inorganic phosphate consequent on anterior pituitary injection is therefore unlikely to be due to stimulation of the gonads with the liberation of gonadal hormone into the blood stream.

(2) Injection of progesterone and oestradiol benzoate into hypophysectomised animals produces a fall of plasma inorganic phosphate similar to that occurring in operative control and normal animals.

(3) The anterior pituitary is therefore not involved in the action of progesterone or oestradiol benzoate on the plasma inorganic phosphate.

(4) The action of progesterone on plasma inorganic phosphate is independent of an associated action on ovulatory processes as ovulatory processes have nothing to do with the fall in plasma inorganic phosphate.

THE INTERRELATIONSHIP BETWEEN

THE PITUITARY, THE PARATHYROIDS AND THE THYROID.

INTER-RELATIONSHIP BETWEEN ANTERIOR PITUITARY PARATHYROIDS AND THYROID.

(a) Parathyroid hormone injection in anterior lobe hypophysectomised toads.

Animals were hypophysectomised according to the method of Hogben (1923). The anterior lobe only was removed. The storage and feeding have been described.

The animals were injected 5 months after operation. No food was given for one week prior to injection. Injections were made into the ventral lymph sac. This route was chosen as being the nearest approach to an intravenous injection obtainable in a toad. Russel and Cori (1937) have shown that the absorption of adrenaline from the subcutaneous site of injection in hypophysectomised rats is diminished. Although intraperitoneal injection is not analogous to subcutaneous injection, absorption has to take place through the peritoneum and endothelium of the blood vessels whereas in the intra-lymphatic injection the lymph drains directly into the veins.

Since normal hypophysectomised and operative control animals showed no change in plasma inorganic phosphate when injected with small quantities of frog Ringer solution (see previous experiments) it was considered unnecessary to inject controls.

Fifty .....

Fifty hypophysectomised animals and 51 operative controls were each injected with 0.25 ml. parathyroid hormone (25 Hanson units). Animals were kept at ordinary room temperature. (16.5°C)

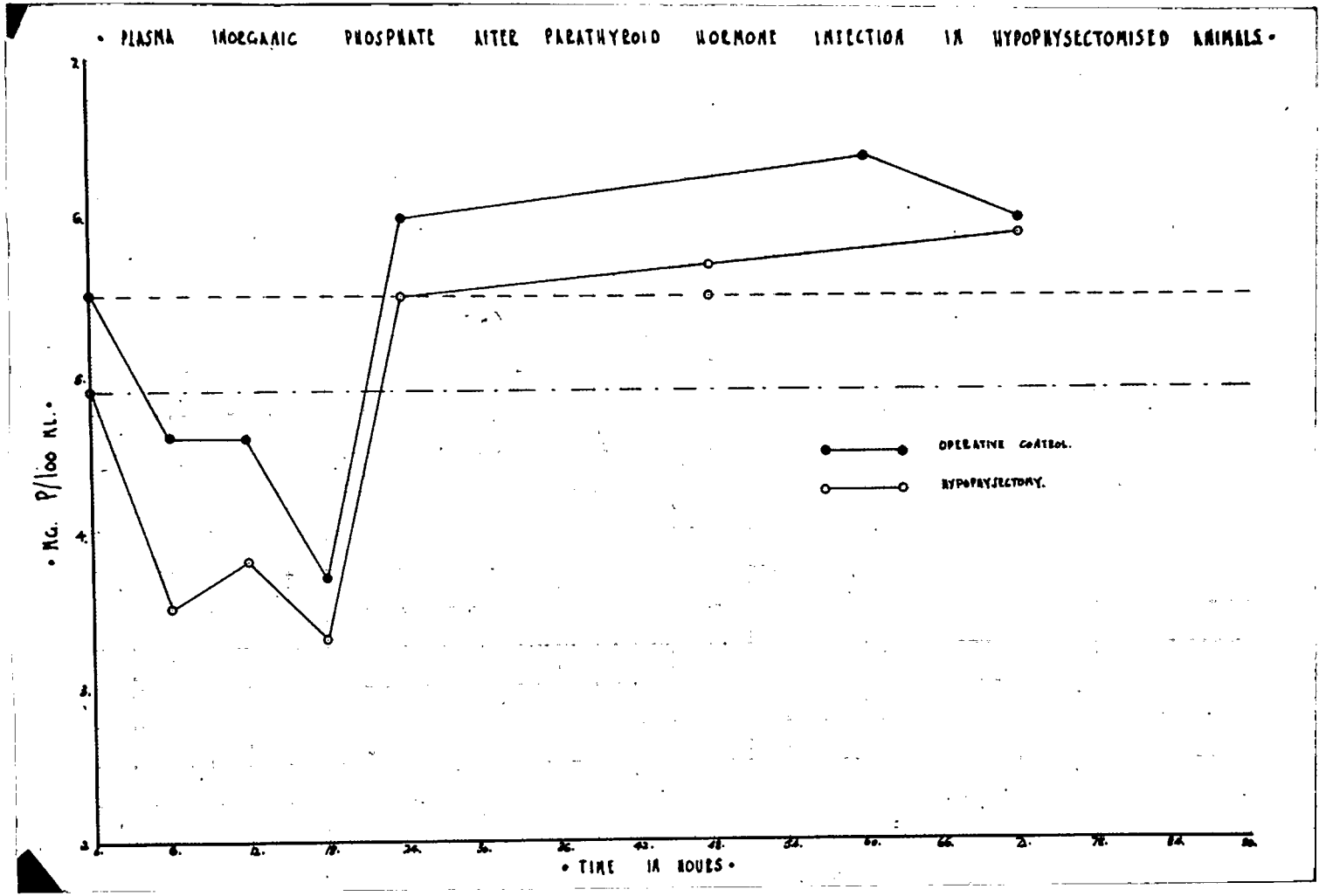
Tables LVII A and LVIIIB and Graph XIX show the effects of parathyroid hormone on anterior lobe hypophysectomised and operative control animals.

TABLE LVII A

Plasma inorganic phosphate after injection of parathyroid hormone (25 Hanson units) into anterior lobe hypophysectomised animals.

Pre-operative normal	4.8 ± 0.13	(8)
5 month hypophysectomy	4.9 ± 0.10	(9)

Time in hours after injection	Parathormone 25 units.
6	3.5 ± 0.14 (4)
12	3.8 ± 0.29 (6)
18	3.3 ± 0.22 (5)
24	5.5 ± 0.15 (7)
48	5.7 ± 0.14 (7)
60	5.9 ± 0.19 (6)
72	5.9 ± 0.13 (6)


• PLASMA INORGANIC PHOSPHATE AFTER PARATHYROID HORMONE INJECTION IN HYPOPHYSECTOMISED ANIMALS •
• MG. P/100 ML. •
• TIME IN HOURS •
OPERATIVE CONTROL.
HYPOPHYSECTOMY.

XIX BMWD

TABLE LVII B.

Plasma inorganic phosphate after injection of parathyroid hormone (25 Hanson units) into operative control animals.

Pre-operative normal	4.8 ±0.13 (8)
5 month operative control	5.5 ±0.21 (4)

Time in hours after injection	Parathormone 25 units
6	4.6 ±0.26 (3)
12	4.6 ±0.35 (3)
18	3.6 ±0.17 (4)
24	6.0 ±0.14 (4)
48	5.5 ±0.18 (4)
60	6.4 ±0.46 (4)
72	6.0 ±0.26 (4)

Each estimation required approximately 2 animals. Detailed figures appear in the appendix:protocol No. 32.

(b) Thyroxine injection in anterior lobe hypophysectomised animals.

The hypophysectomised animals came from the same batch as those used for parathyroid injections.

Animals .....

Animals were injected 5 months after operation and injections were all given via the ventral lymph sac for reasons already given. As in the parathyroid experiments no controls were injected.

Forty three hypophysectomised and 45 operative controls were each injected with 0.5 ml. (0.1 mg.) thyroxine sodium. Animals were kept at ordinary room temperature (16.5°C).

Tables LVIIIA and LVIIIB and Graph XX show the effect of thyroxine sodium on hypophysectomised and operative control animals.

TABLE LVIIIA

Plasma inorganic phosphate after injection of thyroxine sodium (0.1 mg.) into anterior lobe hypophysectomised animals

Pre-operative normal	4.8 ± 0.13	(8)
5 month hypophysectomy	4.9 ± 0.1[illegible]	(9)

Time in hours after injection	Thyroxine Sodium 0.1 mg.
24	4.1 ± 0.22 (6)
48	3.4 ± 0.23 (4)
72	5.1 ± 0.36 (4)
96	5.1 ± 0.16 (6)
144	4.8 ± 0.25 (4)

Table LVIIIB .....

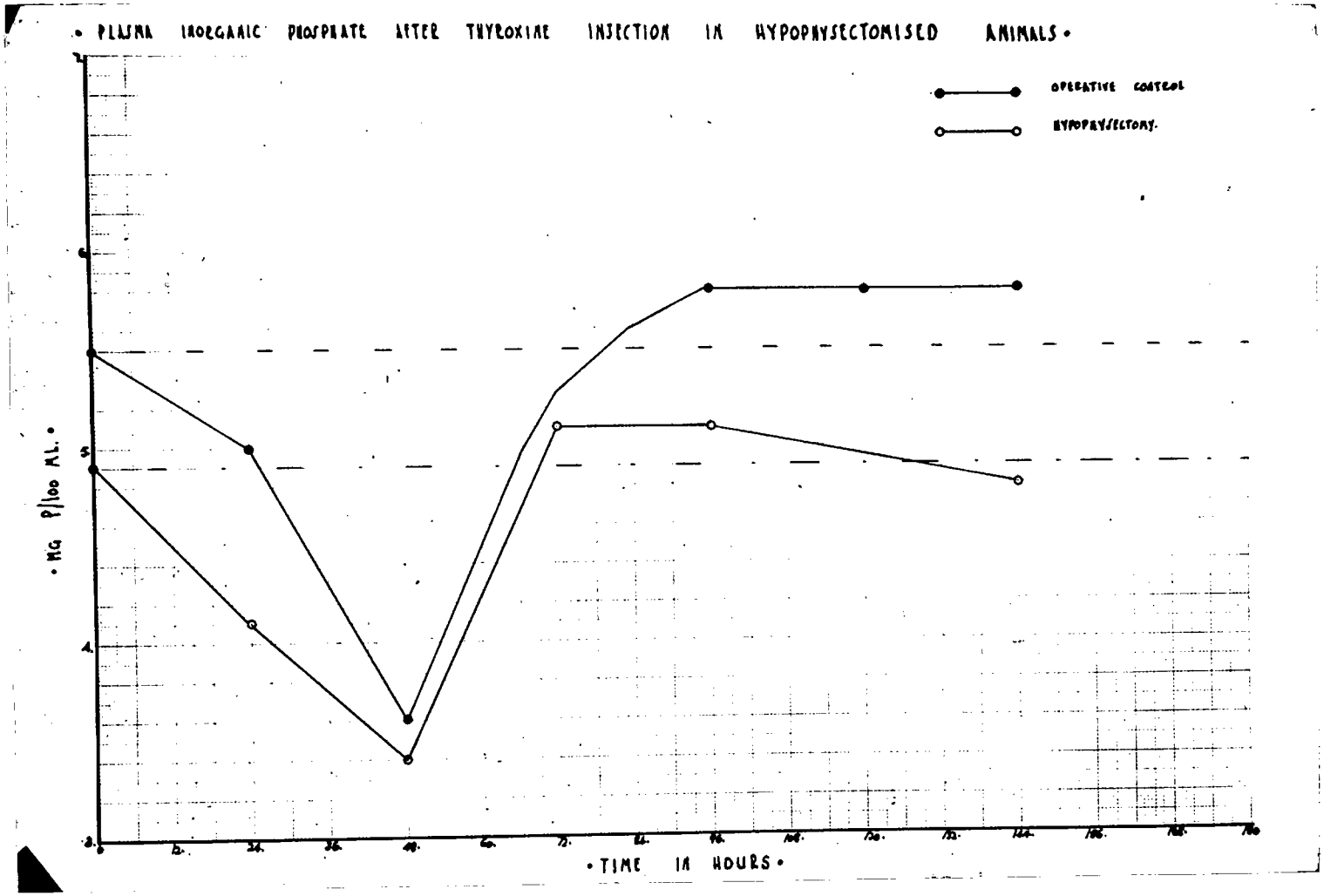

• PLASMA INORGANIC PHOSPHATE AFTER THYROXINE INJECTION IN HYPOPHYSECTOMISED ANIMALS •
OPERATIVE CONTROL
HYPOPHYSECTOMY.
• MG P/100 ML. •
5
4
• TIME IN HOURS •

TABLE LVIIIB.

Plasma inorganic phosphate after injection of thyroxine sodium (0.1 mg.) into operative control animals.

Pre-operative normal	4.6 ± 0.13	(6)
5 month operative control	5.5 ± 0.21	(4)

Time in hours after injection	Thyroxine sodium 0.1 mg.
24	5.0 ± 0.10 (3)
48	3.6 ± 0.32 (4)
96	5.8 ± 0.18 (5)
120	5.8 ± 0.23 (3)
144	5.8 ± 0.40 (2)

Each estimation required approximately 2 animals. Detailed figures appear in the appendix:protocol No. 33.

DISCUSSION .....

DISCUSSION.

(a) Parathyroid hormone injection in anterior lobe hypophysectomised animals.

Hypophysectomy produced a fall in plasma inorganic phosphate (13 per cent).

Hypophysectomised animals injected with parathyroid hormone showed the following changes:-

(4.9 pre-injection level), 3.5, 3.8, 3.3, 5.5, 5.7, 5.9, 5.9.

Operative controls showed the following:-

(5.5 pre-injection level), 4.6, 4.6, 3.7, 6.0, 5.5, 6.4, 6.0.

It will be seen that there is little difference in the response of hypophysectomised or operative controls to parathyroid hormone.

This is well shown in Graph XIX and Table LIX below. Both showed a fall followed by a rise.

TABLE LIX .....

TABLE LIX.

Percentage changes in plasma inorganic phosphate in hypophysectomised and operative controls following injection of parathyroid hormone.

Time in hours after injection	6	12	18	24	48	60	72
Parathyroid hormone into hypophysectomised	-30	-22	-32	+12	+16	+20	+20
Into operative control	-16	-16	-33	+9		+16	+9

Injection of parathyroid hormone has the same effect on hypophysectomised, operative control and normal animals, namely an initial fall in plasma inorganic phosphate followed by a subsequent rise above the normal level.

It is, therefore, probable that the parathyroid hormone acts by directly affecting phosphorus metabolism without the intervention of the anterior pituitary.

The fall in plasma inorganic phosphate following hypophysectomy cannot be attributed to parathyroid deficiency because:-

(a) injections of parathyroid hormone into normal animals produce a fall of plasma inorganic phosphate and an opposite effect might be expected with parathyroid deficiency (see introduction).

(b) injection of parathyroid hormone into anterior

lobe .....

lobe hypophysectomised animals does not restore the plasma inorganic phosphate but produces a further fall.

(b) Thyroxine injections in anterior lobe hypophysectomised animals.

Hypophysectomised animals injected with thyroxine showed the following changes:-

(4.9 pre-injection value), 4.1, 3.4, 5.1, 5.1, 4.8.

Operative controls showed the following:-

(5.5 pre-injection value), 5.0, 3.6, 5.9, 5.8, 5.8.

It will be seen that there is little difference in the response of hypophysectomised or operative controls to thyroxine.

This is well shown in Graph XX and Table LX below.

TABLE LX.....

TABLE LX.

Percentage changes in plasma inorganic phosphate in hypophysectomised and operative controls following injection of thyroxine.

Time in hours after injection	24	48	72	96	120	144
Thyroxine Hypophysectomised	-16	-30	+4	+4		-2
Operative Control	-9	-35		+7	+5	+5

Injection of thyroxine has the same effect on hypophysectomised, operative control and normal animals, namely an initial fall in plasma inorganic phosphate followed by a return to the normal level.

It is therefore probable that the thyroid hormone acts by directly affecting phosphorus metabolism without the intervention of the anterior pituitary.

The fall in plasma inorganic phosphate following hypophysectomy cannot be attributed to thyroid deficiency because:-

(a) thyroid deficiency produces no changes in plasma inorganic phosphate

(b) injection of thyroxine into anterior lobe hypophysectomised animals does not restore the plasma inorganic

phosphate .....

phosphate level but produces a further drop.

Reib and Popper (1936) found that hypophysectomised rats were markedly more susceptible to the action of thyroxine than normal animals. In hypophysectomised animals, the body weight is markedly reduced and the oxygen consumption increased.

Soskin et al., (1939) found that intravenous injection of thyroxine in hypophysectomised dogs maintained a normal blood sugar throughout long period of fasting, but did not diminish the hypersensitivity to insulin. They concluded that the secondary atrophy of the thyroid played an important part in the carbohydrate disturbance in the hypophysectomised animal. In Xenopus laevis injection of thyroxine does not restore the inorganic phosphate level of hypophysectomised animals so that the fall in plasma inorganic phosphate cannot be due to secondary atrophy of the thyroid. If the carbohydrate disturbance in hypophysectomised animals may be explained by thyroid atrophy, then the phosphate changes occur independent of carbohydrate changes. This gives further support to the view that the action of the pituitary on the plasma inorganic phosphate is a direct one on the plasma constituents.

SUMMARY .....

SUMMARY.

(1) Injection of parathyroid hormone has the same effect on the plasma inorganic phosphate of hypophysectomised, operative control and normal animals, namely an initial fall followed by a subsequent rise above the normal level.

(2) It is, therefore, suggested that the parathyroid hormone acts by directly affecting phosphorus metabolism without the intervention of the anterior pituitary.

(3) The fall in plasma inorganic phosphate following hypophysectomy cannot be attributed to parathyroid deficiency because:-

(a) Injections of parathyroid hormone produce a fall in plasma inorganic phosphate and an opposite effect might be expected with hypoparathyroidism (see introduction)

(b) injection of parathyroid hormone into anterior lobe hypophysectomised animals does not restore the plasma inorganic phosphate, but produces a further depression of the level.

(4) Injection of thyroxine has the same effect on plasma inorganic phosphate of hypophysectomised, operative control and normal animals, namely a fall followed by a subsequent return to the normal level.

(5) It is, therefore, suggested that thyroxine acts by directly .....

directly affecting phosphorus metabolism without the intervention of the anterior pituitary.

(6) The fall in plasma inorganic phosphate following hypophysectomy cannot be attributed to thyroid deficiency because:-

(a) thyroid deficiency produces no change in plasma inorganic phosphate

(b) injection of thyroxine into anterior lobe hypophysectomised animals does not restore plasma inorganic phosphate but produces a further depression.

GENERAL DISCUSSION.

(1) The objects of this investigation have been outlined.

(2) A description of the following has been given:-

(a) The method of collection and the storage of the animals.

(b) The collection of blood, the use of anticoagulants and the preparation of heparin.

(c) The biochemical methods of estimation of plasma inorganic phosphate used in this investigation.

(d) The method of anaesthesia and the post-operative care employed.

(3) Hypophysectomy was performed according to the method of Hogben (1923), thyroidectomy according to that of Gitlin (1939a) and gonadectomy according to that of Shapiro (1933b). Pancreatectomy has been described for the first time.

(4) The method of injection, the preparation of anterior pituitary extract and the hormones used in this investigation have been described.

(5) Experiments are described to show that a range of 15°C does not affect the level of plasma inorganic phosphate of the South African clawed toad.(Xenopus laevis)

(6) Whereas the serum calcium level is higher in females than in males throughout the normal reproductive cycle, the plasma inorganic phosphate is higher in females only during the pro-oestrous and anoestrous periods

(7) The .....

(7) The reproductive cycle in females is associated with changes in serum calcium and plasma inorganic phosphate levels and in ovarian activity. In male toads the plasma inorganic phosphate falls slightly during the breeding season just as in female toads, but as this fall is not marked the plasma inorganic phosphate of male and of female toads are the same during the breeding season. The serum calcium of male toads, in contrast, remains remarkably steady.

(8) Captivity first causes a fall, then a steadily maintained elevated plasma inorganic phosphate. Serum calcium first rises then steadily declines in female toads.

(9) It is suggested that seasonal variations in the amount of solar radiation and inadequate illumination during captivity may be important agencies influencing the plasma inorganic phosphate and the serum calcium through the parathyroid glands and the pituitary; and perhaps ovarian activity through the anterior lobe of the hypophesis.

(10) Changes occurring due to seasonal variations and captivity involve several endocrine glands, particularly the pituitary, the parathyroids and the gonads and necessitate investigation of the relation of these glands to plasma inorganic phosphate.

(11) Removal of the anterior lobe of the pituitary (i.e. pars anterior and pars tuberalis) leads to a significantly

lower .....

lower plasma inorganic phosphate level than in operative controls.

(12) When the pars tuberalis regenerates the plasma inorganic phosphate remains low in spite of the return of serum calcium to the normal level.

(13) The progressive fall in plasma inorganic phosphate is due to the removal of the anterior lobe of the pituitary alone.

(14) The fall in plasma inorganic phosphate may be observed within one week of hypophysectomy and is fairly well marked within 2 to 3 weeks.

(15) Injection of anterior pituitary extract into anterior lobe hypophysectomised animals produces a rise of plasma inorganic phosphate to the preoperative level, then a fall below the pre-injection level.

(16) Injection of anterior pituitary extract into normal animals produces an initial rise in plasma inorganic phosphate followed by a fall below the pre-injection level.

(17) The anterior pituitary affects calcium and inorganic phosphate in the same direction and it is probable that these two substances are affected through different mechanisms. The fall in serum calcium following hypophysectomy may be due to hypoparathyroidism, but this cannot explain the fall in plasma inorganic phosphate.

(18) The possible modes of action of the anterior lobe

of .....

of the pituitary are

(a) by altering the plasma constituents

(b) by affecting carbohydrate and consequently phosphorus metabolism.

(c) by stimulating some other gland e.g. the adrenal cortex, the thyroid, the parathyroids, the pancreas or the gonads.

(d) by raising the renal threshold to phosphate, (this would produce a fall of plasma inorganic phosphate on hypophysectomy and opposite effects with anterior pituitary extract injection).

(19) The secondary fall of plasma inorganic phosphate below the pre-injection level after injection of anterior pituitary extract may be due to a reflex stimulation of insulin secretion, by the hyperglycaemia, or a direct stimulation of some other endocrine gland such as the thyroid.

(20) Changes occurring due to hypophysectomy and anterior pituitary extract injection may involve the other endocrine organs and necessitate investigation of the relation of these glands to plasmainorganic phosphate.

(21) Injection of potassium phosphate in amounts equal to the total blood inorganic phosphate content of a toad, produces no effect on the plasma inorganic phosphate, whereas injection of 0.5 ml. fluid produces a slight diluting effect on the plasma.

(22) .....

(22) Intraperitoneal or intralymphatic injection of parathyroid extract into normal females produces an initial fall then a rise in plasma inorganic phosphate above the pre-injection level.

(23) Repeated injections of parathyroid extract produce an elevated plasma inorganic phosphate level which returns to normal 48 hours after the cessation of injections.

(24) The normal physiological action of the parathyroids is probably to produce a fall in plasma inorganic phosphate. The subsequent rise is probably an overdosage phenomenon.

(25) Seasonal variations and captivity effects have been ascribed to changes in parathyroid activity. Results of experiments on the effect of parathyroid extract on plasma inorganic phosphate are in keeping with this hypothesis.

(26) The secondary fall in plasma inorganic phosphate consequent on injection of anterior pituitary extract may possibly be due to stimulation of the parathyroid glands by the extract with the discharge of parathyroid hormone into the blood-stream.

(27) No further light has been thrown on the actual mode of action of parathyroid extract.

(28) Gonadectomy produces no change in plasma inorganic phosphate 6 months after operation in the case of females and 3 months in the case of males. Serum calcium, however,

shows .....

shows a fall 2 weeks after ovariectomy and 6 months after orchidectomy.

(29) It is probable that the changesin calcium is affected partly by the anterior pituitary. The serum calcium and plasma inorganic phosphate are probably affected by different anterior pituitary principles.

(30) Seasonal variations in plasma inorganic phosphate are probably not due to ovarian activity.

(31) Injection of oestradiol benzoate and of progesterone produces a fall in plasma inorganic phosphate. Ovarian extracts produce a rise in serum calcium, but the responsible factor is probably not oestrin nor progesterone.

(32) It is probable that the ovary in the amphibian has no effect on the plasma inorganic phosphate though it may affect the serum calcium either directly or indirectly.

(33) It is probable that the effect of mammalian extract on plasma inorganic phosphate is not strictly comparable with the effect of the animal's own ovaries on phosphorus metabolism. The amphibian ovary probably produces hormones which are not identical with the mammalian hormones, oestradiol and progesterone.

(34) Thyroidectomy produces no change in plasma inorganic phosphate up to 7 months after thyroidectomy.

(35) Injection of 0.1 mg. thyroxine produces a fall of plasma inorganic phosphate in normal animals with a subsequent return to the normal level.

(36) .....

(36) Repeated injection of thyroxine (total dose of 0.4 - 0.6 mg.) produces an elevated plasma inorganic phosphate level which does not return to the normal level within 48 hours after cessation of injections.

(37) Under physiological conditions the thyroid probably produces no change in plasma inorganic phosphate.

(38) The fall in plasma inorganic phosphate following injection of thyroxine is attributed to an increased demand for inorganic phosphate consequent on increased metabolic activity.

(39) The rise in plasma inorganic phosphate following repeated injections of thyroxine is attributed to consumption of carbohydrate stores with consequent diminished demand for inorganic phosphate. Mobilisation of calcium and phosphate from the bones and the soft tissues probably assists this reaction.

(40) The secondary fall of plasma inorganic phosphate consequent on injection of anterior pituitary extract may possibly be due to stimulation of the thyroid gland with the discharge of thyroid hormone into the blood stream.

(41) Ether has no effect on plasma inorganic phosphate 6 hours after anaesthesia.

(42) Pancreatectomy produces a rise in plasma inorganic phosphate which appears within 3 hours after operation.

(43) Injection of insulin into pancreatectomised animals produces a reduction of plasma inorganic phosphate towards

the normal level.

(44) Injection of insulin into normal animals produces a fall in plasma inorganic phosphate with a rise above the pre-injection level when the animals show hypoglycaemic symptoms.

(45) A direct relationship between the pancreas and phosphorus metabolism is suggested.

(46) The effect of insulin is a dual one namely;

(a) a reflex secretion of adrenaline;

(b) formation of an as yet unknown organic compound probably in the muscles.

The two actions combined produce the fall in plasma inorganic phosphate.

(47) Experiments showing the effect of adrenaline on the plasma inorganic phosphate of Xenopus are required to confirm this hypothesis as to the effect of insulin on Xenopus.

(48) The rise of plasma inorganic phosphate during the hypoglycaemic state is ascribed to the cessation of phosphate withdrawal for metabolic purposes and since the call on inorganic phosphate is no longer present the level rises in the blood. Increased excretion of phosphate is not sufficient to prevent a rise of blood inorganic phosphate.

(49) Changes in blood glucose are directly proportional to changes in plasma inorganic phosphate. On pancreatectomy both rise and with insulin injection both fall.

(50) The secondary fall of plasma inorganic phosphate

consequent .....

consequent on injection of anterior pituitary extract may possibly be due to reflex stimulation of the pancreas by the hyperglycaemia with the discharge of insulin into the blood-stream.

(51) Injection of adrenaline into normal animals produces a fall in plasma inorganic phosphate. This is followed by a temporary rise above the pre-injection level.

(52) A direct relationship between adrenaline and phosphorus metabolism is suggested.

(53) The fall in plasma inorganic phosphate is attributed to withdrawal of inorganic phosphate for the formation of hexose phosphate. The accumulation of hexose phosphates in the body is sufficient to account for the fall in blood and urinary inorganic phosphates.

(54) The subsequent rise in plasma inorganic phosphate is attributed to an overswing phenomenon. The initial action of adrenaline is to produce a fall in inorganic phosphate and a rise in calcium follows. The H ion concentration is upset and an acidosis results. This is followed by a slow compensatory phase in which the inorganic phosphate of both blood and urine rise and calcium falls. An overswing occurs leading to a transient rise of plasma inorganic phosphate above the normal level.

(55) Reflex secretion of adrenaline after insulin injection may contribute to the fall in plasma inorganic phosphate occurring after the injection of insulin.

(56) .....

(56) Injection of insulin into hypophysectomised animals produces a decreased diminution of plasma inorganic phosphate as compared with operative controls. It is suggested that the cause is the inability of adrenaline, reflexly secreted to assist in lowering the plasma inorganic phosphate. This may possibly be due to abnormal stability of muscle glycogen and consequently of the organic phosphorus compounds.

(57) Insulin depresses plasma inorganic phosphate partly by a direct action on phosphorus metabolism. The complete effect in normal animals is a dual one:-

(a) reflex secretion of adrenaline

(b) formation of an as yet unknown compound.

The two actions combined produce the whole fall in plasma inorganic phosphate. In hypophysectomised animals the action of adrenaline is cancelled out so that only the formation of organic compound takes place and as a result the demand on inorganic phosphate is less than in normal animals where both mechanisms are at work.

(58) The anterior pituitary is necessary for the complete action of adrenaline on plasma inorganic phosphate. Absence of the anterior pituitary leads to no initial fall of plasma inorganic phosphate after adrenaline injection.

(59) This is attributed to an abnormal stability of muscle glycogen tothe mobilising action of adrenaline. Small changes in hexose phosphate occur and consequently little extra demand for inorganic phosphate takes place.

(60) .....

(60) The secondary rise in plasma inorganic phosphate after adrenaline injection is presumably not due to changes in muscle hexose phosphate but may possibly be due to slow compensatory processes adjusting the relationships of the various plasma constituents. The anterior pituitary is not required for this.

(61) Injection of anterior pituitary extract into ovariectomised animals produces a fall in plasma inorganic phosphate similar to that occurring in operative control animals. The secondary fall in plasma inorganic phosphate consequent on anterior pituitary injection is therefore unlikely to be due to stimulation of the gonads with the liberation of gonadal hormone into the blood stream.

(62) Injection of progesterone and oestradiol benzoate into hypophysectomised animals produces a fall of plasma inorganic phosphate similar to that occurring in operative controls and in normal animals.

(63) The anterior pituitary is therefore not involved in the action of progesterone or oestradiol benzoate on the plasma inorganic phosphate.

(64) The action of progesterone on plasma inorganic phosphate is independent of an associated action on ovulatory processes as ovulatory processes have nothing to do with the fall in plasma inorganic phosphate.

(65) Injection of parathyroid hormone has the same effect on the plasma inorganic phosphate of hypophysectomised,

operative .....

operative control and normal animals, namely an initial fall followed by a subsequent rise above the normal level.

(66) It is therefore suggested that the parathyroid hormone acts by directly affecting phosphorus metabolism without the intervention of the anterior pituitary.

(67) Injection of thyroxine has the same effect on plasma inorganic phosphate of hypophysectomised, operative control and normal animals, namely a fall followed by a subsequent return to the normal level.

(68) It is therefore suggested that thyroxine acts by directly affecting phosphorus metabolism without the intervention of the anterior pituitary.

(69) The fall in plasma inorganic phosphate following anterior lobe hypophysectomy cannot be due to hypofunction of the pancreas because:-

(a) pancreatic deficiency leads to a rise in plasma inorganic phosphate;

(b) injection of insulin into anterior lobe hypophysectomised animals does not restore the plasma inorganic phosphate level but produces a further depression. It may possibly be due to the unopposed action of insulin due to lack of the anti-insulin factor from the pituitary gland.

(70) The fall in plasma inorganic phosphate following anterior lobe hypophysectomy cannot be due to hypofunction of the

adrenal .....

adrenal medulla because:-

(a) adrenaline produces a fall in plasma inorganic phosphate.

(b) injection of adrenaline into anterior lobe hypophysectomised animals does not restore the inorganic phosphate level.

(71) It cannot be due to hypofunction of the gonads because:- hypofunction of the gonads produces no change in plasma inorganic phosphate.

(72) It cannot be due to parathyroid deficiency either because:-

(a) injection of parathyroid hormone produces a fall in plasma inorganic phosphate and an opposite effect might be expected with hypoparathyroidism. (see introduction page

(b) injection of parathyroid hormone into anterior lobe hypophysectomised animals does not restore the plasma inorganic phosphate but produces a further depression.

(73) It cannot be due to thyroid deficiency because:-

(a) thyroid deficiency produces no change in plasma inorganic phosphate;

(b) injection of thyroxine into anterior lobe hypophysectomised animals does not restore the plasma inorganic phosphate but produces a further depression.

(74) Possible modes of action of the anterior pituitary on

the plasma inorganic phosphate are therefore:-

(a) by altering the plasma constituents.

(b) by affecting carbohydrate and consequently phosphorus metabolism either directly or through the adrenal cortex.

(c) by raising the renal threshold to phosphate.

(75) It is considered most probable that the pituitary directly affects the plasma constituents and the presence of the anterior lobe is necessary for the maintenance of a normal plasma inorganic phosphate level.

GENERAL SUMMARY.

(1) Temperature changes from 5°C to 23°C does not affect the level of the plasma inorganic phosphate of the South African clawed toad.

(2) The plasma inorganic phosphate level of females is higher than that of males during the pro-oestrous and anoestrous periods.

(3) The plasma inorganic phosphate of male and female toads is highest in the summer, autumn and early winter and lowest in late winter and early spring i.e. during the rainy and breeding season.

(4) Captivity first causes a fall, then a steadily maintained elevated plasma inorganic phosphate.

(5) Removal of the anterior lobe of the pituitary (i.e. pars anterior and pars tuberalis) leads to a significantly lower plasma inorganic phosphate level than in operative controls.

(6) When the pars tuberalis regenerates, the plasma inorganic phosphate remains depressed.

(7) The progressive fall of plasma inorganic phosphate is due to removal of the anterior pituitary alone. The effect of hypophysectomy may be observed within one week after operation.

(8) Injection of acid extracts of anterior pituitary into anterior lobe hypophysectomised animals produces a rise

of plasma inorganic phosphate to the preoperative level then a fall below the pre-injection level.

(8) Injection of acid extracts of anterior pituitary into normal animals produces an initial rise in plasma inorganic phosphate followed by a fall below the pre-injection level.

(9) Injection of potassium phosphate in amounts equal to the total blood inorganic phosphate content of a toad produces no effect on the plasma inorganic phosphate, whereas injection of 0.5 ml. fluid produces a slight diluting effect on the plasma.

(10) Intraperitoneal or intralymphatic injection of parathyroid extract into normal animals produces an initial fall then a rise in plasma inorganic phosphate above the pre-injection level.

(11) Repeated injections of parathyroid extract into normal animals produce an elevated plasma inorganic phosphate level.

(12) Gonadectomy produces no change in plasma inorganic phosphate.

(13) Injections of progesterone and of oestradiol benzoate into normal animals produce a fall in plasma inorganic phosphate.

(14) Thyroidectomy produces no change in plasma inorganic phosphate.

(15) Injection of thyroxine into normal animals produces a fall in plasma inorganic phosphate.

(16) Repeated injection of thyroxine into normal animals

produce a rise in plasma inorganic phosphate.

(17) Pancreatectomy produces a rise in plasma inorganic phosphate and blood glucose within a few hours after operation.

(18) Injection of insulin into pancreatectomised animals produces a reduction of plasma inorganic phosphate towards the normal level.

(19) Injection of insulin into normal animals produces a fall in plasma inorganic phosphate and blood glucose. The plasma inorganic phosphate rises above the pre-injection level when the animals show hypoglycaemic symptoms.

(20) Injection of adrenaline into normal animals produces a fall in plasma inorganic phosphate. This is followed by a temporary rise above the pre-injection level.

(21) Injection of insulin into anterior lobe hypophysectomised animals produces a decreased diminution of plasma inorganic phosphate as compared with operative controls.

(22) Injection of adrenaline into anterior lobe hypophysectomised animals produces no initial fall in plasma inorganic phosphate.

(23) Injection of acid extracts of anterior pituitary into ovariectomised animals produces a secondary fall in plasma inorganic phosphate similar to that occurring in operative control animals.

(24) Injection of progesterone and of oestradiol benzoate

into .....

into hypophysectomised animals produces a fall in plasma inorganic phosphate similar to that occurring in operative controls.

(25) Injection of parathyroid extract in hypophysectomised animals has the same effect on the plasma inorganic phosphate as in operative control animals.

(26) Injection of thyroxine in hypophysectomised animals has the same effect on the plasma inorganic phosphate as in operative control animals.

AVENUES FOR FURTHER STUDY.

(1) Investigations on the effect of the gonadotrophic free fraction of anterior pituitary extract on the plasma inorganic phosphate.

(2) The effect of anterior pituitary extract on the plasma inorganic phosphate of pancreatectomised and thyroidectomised animals.

These experiments will throw more light on the mode of action of the anterior pituitary.

(3) The effect of posterior pituitary extracts on the plasma inorganic phosphate.

(4) Injection of Xenopus ovarian extracts.

No active extract of Xenopus ovaries has yet been prepared.

(5) Injection of oestradiol and progesterone in castrated animals.

These experiments will finally determine the effect of the Xenopus ovary on plasma inorganic phosphate and also will show whether the mammalian hormones act directly on phosphorus metabolism or through the intermediation of the animal's own ovaries.

(6) The effect of thyroxine on the plasma inorganic phosphate of thyroidectomised animals.

(7) .....

(7) The comparison between intralymphatic and intraperitoneal injections of insulin and adrenaline in normal and in hypophysectomised animals.
This will settle the question of the decreased fall in plasma inorganic phosphate after insulin and after adrenaline injection in hypophysectomised animals.
(8) The effect of adrenal cortex extract on the plasma inorganic phosphate of normal and hypophysectomised animals.

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PROTOCOL NO. 1.

ANTERIOR PITUITARY EXTRACTS.

	S.A.P.1A	S.A.P.2A	S.A.P.3A	S.A.P.4A
No. of Anterior Lobes	38	21	12	16
Weight of tissue	15.7	11.57	7.384	11.406
Amount of Extract (ml.)	38	21	12	23
Amt. of A.P.E./animal (gm.)	0.21	0.28	0.31	0.25

	S.A.P.1B	S.A.P.2B
No. of Anterior Lobes	36	48
Weight of tissue	13.81	28.5
Amount of Extract (ml.)	18.6	38.4
Amt. of A.P.E./animal (gm.)	0.19	0.30

EFFECT OF TEMPERATURE.

No. of days in tanks	Refrigerator	Thermostat.
7	4.9	5.5
	6.8	5.6
		5.6
		5.2
	Av. 5.8	Av. 5.5
10	6.0	5.8
	6.8	6.6
	7.2	6.0
		5.9
	Av. 6.7	Av. 6.1
14	5.2	6.9
	5.2	6.9
	4.9	6.8
		6.3
	Av. 5.1	Av. 6.7

EFFECT OF CAPTIVITY.

	Normal 16th March	2 mth 10th May	3 mth 10th June	4 mth 10th July	5 mth 10th Aug.	6 mth 10th Sept.	7 mth 10th Oct.	8 mth 16th Nov.	9 mth 14th Dec.
	6.0	4.2	4.2	6.3	4.7	5.0	7.3	7.6	5.1
	6.5	4.1	4.5	6.4	4.7	4.9	6.3	5.1	5.3
	7.7	5.1	4.8	4.5	4.6	5.0	6.5	6.1	6.4
	8.3	4.9	4.6	4.6		4.9	5.8	4.8	5.8
	8.0	4.9		4.4			6.3		
	7.4	4.9		4.3			6.3		
	7.5	4.9		4.2			7.9		
	7.9						7.5		
	7.3								
AVERAGE	7.6	4.7	4.5	4.9	4.7	5.0	6.7	5.9	5.6
No. of fœcis	18	8	8	9	7	8	11	9	9
Body Wt. (gm.)		368.6	433	390	284.5	331.2	389.8	359	281.5
Ovary Wt.		32.5	29	29	23	31	28	33	16
Gonad Ratio		8.8	6.7	7.4	8	9.4	7.2	9.2	5.7

RUBCO

Effect of captivity
Cont.

	10 mths January 13	11 mths February 10	12 mths March 10
	5.6	6.3	4.6
	5.1	6.3	4.6
	6.5	5.4	7.3
		7.8	6.7
		6.3	7.4
AVERAGE	5.7	6.4	6.1
No. of toads	11	7	8
Body Wt. (gms)	366.2	267.9	253.6
Ovary weight	23.2	17.5	18.2
Gonad Ratio	6.3	6.5 6.5	7.0

	Normals	3 week captivity	5 week captivity
	6.0	5.3	5.5
	7.4	5.6	5.1
	7.6	5.7	
	7.6	6.4	
		6.3	
		6.2	
AVERAGE	7.2	5.9	5.3

	Normals	3 week captivity
	5.3	4.5
	6.8	4.7
	6.6	
	8.2	
	7.2	
	7.6	
	8.2	
AVERAGE	7.1	4.6

Seasonal variations females.

Dec. 1937	Jan. 1938	Feb. 8th	March 25th	April 20th	May 18th	June 19th	July 20th	Aug. 19th	Sept. 17th	Oct. 18th	Nov. 18th
6.0	7.4	7.2	6.9	6.1	6.9	7.3	4.4	4.6	4.4	5.0	4.7
		7.3									
6.8	7.5	7.3	7.3	5.6	7.3	6.7	4.4	4.8	5.1	5.5	5.7
		7.2									
6.3	7.8	6.4	6.7	5.9	6.6	6.5	4.6	5.1	3.9	4.9	6.3
		6.7									
6.7	7.0	6.7	7.4	6.0	7.0	6.8	4.6	5.0	3.9	4.6	7.5
		7.3									
7.4	6.8	6.8		6.1	6.7	7.0	4.7	5.2	4.7	5.1	5.1
		7.6									
	6.8	7.2		6.0	5.9	6.3	4.3	4.2	4.1	5.6	6.2
		7.5									
	6.8	6.0		6.1	6.4	7.0	4.2	3.9		5.2	6.2
		6.9									
		5.9				7.2	4.7	4.2			7.7
		6.8									
		6.2				6.9	4.7	4.5			6.9
		6.0									
		6.6				6.8		4.6			5.8
		6.6									
		7.6				7.0		4.6			6.5
		7.1									
		7.1						4.3			7.1
		7.3									
		7.6						4.0			
		7.7									
		7.5						3.9			
								4.3			
AVERAGE 6.6	7.2	7.0	7.1	6.0	6.7	6.9	4.5	4.5	4.4	5.1	6.3
No. of toads 9	12	37	10	13	13	13	12	14	15	15	12
Temperature			22.5 c	20c		17 c		15.5c	18.5c	17.5c	18 c

PROTOCOL NO. 14.

VI.

Seasonal Variations Males.

Feb.	April	May	June	July	Aug.	Sept.	Nov.
5.8	5.5	5.3	5.6	4.0	4.3	4.9	4.9
5.7							
5.7	5.5	5.6	6.0	4.1	4.6	4.6	4.0
6.0							
5.6	5.6	5.8	5.9	4.3	4.7	4.5	5.7
5.5							
5.6	5.3	5.8	5.6	4.7	4.8	4.4	4.7
5.9							
6.3	5.3	6.3	5.5	4.8	5.4	4.0	6.5
5.6							
6.6	5.1		5.8	4.1	5.3	4.6	
6.2							
5.9	5.5		5.8	4.3		4.6	
5.7							
6.1				4.6			
6.0							
5.9				4.7			
5.7							
6.4							
6.0							
AVERAGE 5.9	5.4	5.8	5.7	4.4	4.8	4.5	5.2
No. of toads 37	15	13	12	17	17	16	18
Temperature	20°C		17°C		15.5°C	18.5°C	18°C

Effect of Hypophysectomy

	Normal	2 months Total	2 months Ant.Lobe	2 months Op.Cont	3 months Total	3 months Ant.L	3 months Op.C	4 months Total	4 months Ant.L	4 months Op.C
	5.5	4.9	4.0	6.9	3.4	3.1	7.1	4.1	3.9	6.3
	6.6	5.3	5.0	7.1	3.9	4.6	7.5	3.8	4.1	5.8
	6.6		(7.8)	8.0	(5.1)	4.1		3.5	4.6	6.4
	6.0									
	6.1									
AVERAGE	6.2	5.1	5.6	7.3	4.1	3.9	7.3	3.8	4.1	6.2

PHYSIOL. MON. 4.

	5 months Total	5 months Ant.L	5 months Capt.	6 months Total	6 months Ant.L	6 months Capt.	7 months Total	7 months Ant.L	7 months Capt.	8 mths Ant.L	9mths Ant.L
	4.9	3.8	5.0	3.7	3.4	4.0	3.5	2.5	4.6	1.9	1.5
	5.2	3.8	5.0	2.6	2.2	4.6	4.7	2.5	5.0	1.9	1.5
	4.2	3.1	4.6	2.8	3.0	3.5	4.3	3.5		1.9	1.9
	4.6	3.5	4.9	3.1	3.5	3.9		3.6		1.9	1.9
					3.5			4.1			1.6
								3.7			
AVERAGE	4.7	3.6	4.9	3.1	3.1	4.0	4.2	3.3	4.8	1.9	1.7

VIII.

Effect of Hypophysectomy.

3 days Ant.L.	Op.C.	4 days Ant.L.	7 days Total	Ant.L.	Op.C.	8 days Op.C.	Total	14 days Ant.L.	Op.C.	21 days Ant.L.	Total	Hypt.
4.6	5.0	4.5	3.3	4.7	6.3	4.8	5.1	4.5	5.4	3.2	3.5	5.3
4.5	5.5	5.4	4.5	5.6	5.9	6.4	3.6	4.5	6.0	3.5	3.2	5.6
4.2		5.4	3.2	5.5	4.3	6.6	4.3	4.5	5.6	3.8	3.5	5.7
		4.9	4.7	4.9	4.9		4.8	4.7	5.5	3.6	3.5	6.4
		4.9	5.6	5.0	5.0			4.7		3.5	3.3	6.3
			5.6		6.8			4.6				6.2
			4.9		6.4							
Av. 4.4	5.3	5.0	4.5	5.1	5.7	5.9	4.5	4.5	5.6	3.5	3.4	5.9

PROTOCOL NO. VII.

Effect of inorganic phosphate injection

	24 hours		48 hours	
	Phosphate	Control	Phosphate	Control
	5.2	5.6	5.1	4.6
	5.2	5.7	5.5	4.9
	5.5	5.9	5.6	5.2
	5.5	5.8	5.4	5.1
			5.4	6.1
			5.6	5.0
				4.9
AVERAGE	5.3	5.7	5.4	5.1
No. of toads	6	7	6	7

	6 hours			15 hours	
	Normal	Phosphate	Control	Phosphate	Control
	7.0	6.9	6.1	6.9	7.2
	6.6	5.9	5.5	6.4	7.3
	7.1	6.4	5.4	6.6	6.5
	6.5	6.5	5.9		6.5
	6.7	6.6	5.9		7.8
	6.6	6.1			7.3
		6.0			
		6.3			
AVERAGE	6.6	6.3	5.6	6.6	7.1
No. of toads	8	8	8	5	7

PROTOCOL NO. 8.

Effect of S.A.P.2A in hypophysectomised animals.

	Preoperative Normal	2 month Hypophys.	6 Hour S.A.P.	6 Hour Cont.	9 Hour S.A.P.	9 Hour Cont.
	6.0	4.2	4.6	4.1	4.9	4.0
	6.8	4.1	4.6	4.4	5.1	4.1
	6.3	4.2	5.0	4.3	5.6	4.6
	6.7		5.1	4.2	5.9	4.8
	7.4	3.9	5.2	4.3	5.9	4.1
			5.2		6.0	4.3
AVERAGE	6.6	4.1	5.0	4.3	5.6	4.3
No.of toads	9	7	6	7	7	7

	12 Hour S.A.P.	12 Hour Cont.	18 Hour S.A.P.	18 Hour Cont.	24 Hour S.A.P.	24 Hour Cont.	48 Hour S.A.P.	48 Hour Cont.
	5.8		4.8		4.2	5.1	5.6	5.2
	5.7	ESCAPED	5.8	ESCAPED	4.4	4.7	5.7	4.6
	6.3		5.0		4.5	5.1	4.3	5.3
	6.6				4.8	4.6	3.8	4.4
	(7.6)				4.6			
AVERAGE	6.1		5.2		4.5	4.9	4.9	4.9
No.of toads	7		7		7	7	7	7

PROTOCOL NO. 9.

Effect of S.A.P.3A and S.A.P.4A on hypophysectomised animals.

	2 months Hypophys.	12 Hours S.A.P.	Cont.	24 Hours S.A.P.	Cont.	36 Hours S.A.P.	Cont.	48 Hours S.A.P.	Cont.
	5.7	5.8	4.7	6.1	4.4	3.1	3.5	2.9	5.0
	4.9	5.7	4.6	5.3	3.9	2.6	3.2	2.9	5.7
	5.6	6.0	4.8	5.7	4.2	3.0	3.7	3.0	4.5
		6.3	4.5		4.5	3.3	3.9	3.2	
								3.9	
AVERAGE	5.4	6.0	4.7	5.7	4.3	3.0	3.6	3.2	5.1

	2 months Hypophys.	6 Hours S.A.P.	Cont.	12 Hours S.A.P.	Cont.	36 Hours S.A.P.	Cont.
	5.5	5.6	4.1	4.4	4.3	3.5	4.0
	4.2	4.6	4.1	4.7	4.3	4.0	5.5
	4.9	7.7	3.9	4.9		4.1	4.5
	4.5	6.7	3.9			3.6	
AVERAGE	4.8	6.2	4.0	4.7	4.3	3.8	4.7

PROTOCOL NO. 10.

Effect of S.A.P.1A in normal animals.

Normal	S.A.P.1A Brain Ext.	6 Hour S.A.P.	Cont.	10 Hour S.A.P.	Cont.	12 Hour S.A.P.	Cont.
6.1	10.8	5.4	6.7	6.1	6.6	5.5	7.5	6.2
6.6	12.4	6.3	7.1	6.4	6.9	5.5	7.5	6.6
6.0		6.2	7.3	6.4	7.0	5.2	7.4	6.9
5.9			7.1	5.8	7.0	6.2	7.6	7.3
6.1			7.3	5.8	6.5	5.8	6.5	6.4
6.1			7.0	5.9	6.8		7.4	6.2
			7.5		7.1			7.0
			7.1		7.3			
AVERAGE 6.1	11.6/100ml.	6.0/100ml	17.1	6.1	6.9	5.6	7.3	6.7
No. of toads 11			8		8	8	8	8

	18 hour		26 hour		36 hour		50 hour		60 hour	
	S.A.P.	Cont.	S.A.P.	Cont.	S.A.P.	Cont.	S.A.P.	Cont.	S.A.P.	Cont
	5.4	6.5	5.8	6.7	5.4	5.9	5.5	5.9	6.6	6.8
	5.6	6.4	5.6	6.0	4.7	5.4	5.5	6.3	6.2	6.8
	5.3	6.4	5.4	5.9	5.0	6.2	4.7	6.1	6.7	6.9
	6.0	6.1	5.1	5.9	5.0	5.4	4.9	6.6	6.3	6.6
	6.4	6.1	5.1	6.0	5.5	6.2	5.7	6.1	6.4	
	6.1	6.0	4.9	5.9	5.4	6.3	5.5	5.8		
	6.5		4.8	5.7	5.6	5.9	5.8			
						6.4	5.5			
							5.5			
AVERAGE	5.9	6.3	5.2	6.0	5.2	6.0	5.4	6.1	6.4	6.8
No. of toads	8	8	8	8	8	8	8	8	8	8

PROTOCOL NO. 11.

Effect of intraperitoneal parathyroid injection.

	Normal	6 Hours Parathor.	18 Hours Parat.	Cont.	24 Hours Parathor.	36 Hours Parathor
	4.0	4.0	5.1	4.8	6.8	6.1
	4.3	4.4	5.4	4.6	6.5	6.4
	5.0	3.7	5.8	4.8	6.9	7.1
	4.8	3.8	6.0	5.2	7.4	6.3
	4.9	4.5	5.4		6.6	6.2
	5.0		5.8		6.4	6.9
AVERAGE	4.7	4.1	5.6	4.8	6.8	6.5
No. of toads	12	9	9	9	9	7
Temperature	19°C	20°C	19°C	19°C	19°C	19.5°C

Effect of intraperitoneal parathyroid injection (cont.)

	48 Hours Parat.	Cont.	60 Hours Parathormone	72 Hours Parathormone	Control
	6.0	5.2	6.5	6.9	5.1
	6.1	5.3	7.0	7.1	5.4
	6.8	5.5	7.9	6.8	
	6.5		7.6	7.1	
	5.6		7.0	6.2	
	5.1		6.9	6.4	
	6.0				
	6.7				
Average	6.1	5.3	7.2	6.6	5.2
No. of toads	9	9	8	9	10
Temperature	19.5°C	19.5°C	19.5°C	19.5°C	19.5°C

PROTOCOL NO. 12.

Effect of intralymphatic parathyroid injection.

	Normal	6 Hours Parat.	6 Hours Cont.	12 Hours Parat.	12 Hours Cont.	18 Hours Parat.	18 Hours Cont.
	5.4	4.7	4.8	4.3	4.9	5.1	4.1
	5.0	4.7	4.9	4.1	4.7	5.6	4.8
	4.0	5.1	5.5	4.0	4.9	5.3	5.6
	5.0	5.3	5.7	4.1	5.3	4.9	5.5
	5.2	4.2		4.2	5.3	5.5	5.1
		4.6				4.9	4.5
AVERAGE	4.9	4.7	5.2	4.1	5.0	5.2	4.9
No. of toads	11	7	6	5	7	6	7
Temperature	19°C	19°C	19°C	19°C	19°C	19°C	19°C

Effect of intralymphatic parathyroid injection (cont.)

	24 Hours		36 Hours		48 Hours		72 Hours	
	Parat.	Cont.	Parat.	Cont.	Parat.	Cont.	Parat.	Cont.
	5.2	5.2	6.0	5.2	4.8	5.2	4.8	4.8
	5.2	4.9	5.4	4.4	5.4	4.6	4.5	4.6
	5.5	4.8	5.4	4.8	5.1	4.6	4.9	5.0
	5.3	4.8	5.2	4.9	4.7	4.9	5.2	
	5.4	5.3	5.7	4.9	5.0		4.8	
		5.1			4.4			
		4.9			5.6			
					5.1			
AVERAGE	5.3	5.0	5.6	4.8	5.0	4.8	4.8	4.8
No. of toads	7	7	4	7	14	7	6	7
Temperature	19°C	19°C	18°C	18°C	21°C	19°C	21°C	21°C

PROTOCOL NO. 13.

Effect of repeated injections of parathyroid.

	Normal	24 Hours		48 Hours	
		Parathormone	Control	Parathormone	Control
	5.4	5.6	4.3	4.5	4.5
	5.0	5.8	5.0	4.7	5.4
	4.0	6.5	4.7	5.9	5.6
	5.0	5.9	5.5	6.6	
	5.2	6.3		4.3	
		6.3		5.4	
AVERAGE	4.9	6.1	4.9	5.2	5.2
No. of toads	11	8	8	8	9

xlx.

PROTOCOL NO. 14.

Effect of ovariectomy.

Normal	1 month Ovari.Op.C.		2 months Ovari.Op.C.		3 months Ovari.Op.C.		4 months Ovari.Op.C.		5 months Ovari.Op.C.		6 months Ovari.Op.C.	
4.6	4.6	5.0	5.0	5.4	5.2	5.0	4.8	5.1	4.5	4.1	3.5	3.7
4.8	5.1	5.6	4.8	5.0	5.0	5.1	5.7	5.5	4.7	5.4	4.5	4.1
4.3	5.8	5.7	4.9	4.4	5.2	5.3	5.9	6.2	5.3	5.1	3.7	4.6
4.8	5.0		5.2	5.4	5.3	5.6	5.5	5.9	5.4	5.2	4.6	4.0
4.8	5.0		5.8	5.1	5.5	5.5	5.4	5.7	5.4			
4.9	6.7		5.2	4.7	5.3	5.5	5.1					
4.6	6.5		4.9	6.1	4.8	5.5	5.7					
4.5			5.4	6.1	4.9	5.7						
4.5			6.2		4.9							
5.3			5.9									
4.8												
AVERAGE 4.7	5.5	5.4	5.3	5.3	5.1	5.4	5.4	5.7	5.1	5.0	4.1	4.1
No. of toads 15	9	8	10	10	8	9	9	10	10	10	10	10
Temp. 22°C	17°C		18°C		19.5°C		21°C		22.5°C		22°C	

PROTOCOL NO. 15.

Effect of ovariectomy.

	24 Hours	
	Ovariectomised	Operative Controls
	6.6	6.1
	6.0	6.4
	6.5	6.5
AVERAGE	6.4	6.3
No. of toads	7	8
Temperature	14.5°C	15°C

Effect of castration.

→ 3

	1 Month		3 Months	
	Castrates	Operative Cont.	Castrates	Operative Cont.
	4.3	4.1	4.3	4.9
	4.6	5.0	5.2	4.9
	4.3	4.9	4.3	4.4
		5.2		4.6
AVERAGE	4.4	4.8	4.8	4.7
No. of toads	8	8	6	7
Temperature	17°C		19.5°C	

PROTOCOL NO. 16.

Effect of progesterone on normal females.

	Normal	6 Hours Progest. Cont.		12 Hours Progest. Cont.		18 Hours Progesterone	24 Hours Progest. Cont.	
	5.1	5.9	4.8	5.1	5.4	4.5	5.3	5.2
	5.1	5.3	4.7	6.2		5.8	5.2	5.7
	5.0	5.7	5.0	5.1		5.4	6.1	
	5.1	4.4				5.9	5.9	
	5.1	4.2				6.0		
	5.0							
AVERAGE	5.1	5.1	4.8	5.5	5.4	5.5	5.6	5.5
No. of toads	12	8	8	7	7	7	7	7
Temperature	16°C	18°C		20°C		20°C	18°C	
Ovulation Count		0,0,0 0,0,0 1,0		0,0,0 0,1 0,0		0,3 2,1,1 2,2	6,6,5 5,4 4,4	

XXI.

Effect of progesterone on normal females. (cont.)

	39 Hours Progest. Cont.		48 Hours Progest. Cont.		60 Hours Progest. Cont.		72 Hours Progest. Cont.		96 Hours Progest. Cont.	
	4.1	5.5	4.4	5.1	4.6	5.0	3.1	4.5	4.7	5.9
	4.2	5.5	4.2	4.8	4.2	4.8	4.2	5.4	4.4	5.5
	4.1	4.3	4.4	5.5	4.9	4.5	4.3	5.3	4.0	5.3
			4.1	5.0	4.5	5.0	3.6	5.3		
			4.2	5.1						
AVERAGE	4.1	5.1	4.3	5.1	4.5	4.8	3.8	5.1	4.4	5.5
No. of toads	7	7	7	7	7	7	7	7	7	8
Temperature	18°C		20°C		20°C		18°C		20°C	
Ovulation Count	5.0 5,1 3,1,4		0,5 6,6 4,5,5		6.3 4,4 1,4,4		6.6 6,6 6,5,5		4.0 0,0 0,0,0	

PROTOCOL NO. 17.

Effect of oestradiol benzoate on normal females.

	Normal	6 Hours Oestrad.Cont.		12 Hours Oestrad.Cont.		18 Hours Oestrad.Cont.	24 Hours Oestrad.Cont.	
	5.1	4.9	4.8	4.6	5.4	4.4	5.9	5.2
	5.1	4.8	4.7	5.0		4.6	5.8	5.7
	5.0	4.4	5.0			4.5		
	5.1	4.6		5.2		5.2	6.0	
	5.0	4.3					5.4	
	5.1	4.5					5.5	
	5.1	4.3					5.8	
AVERAGE	5.1	4.5	4.8	4.9	5.4	4.7	5.7	5.5
No. of toads	12	8	8	7	7	7	7	7
Temperature	16°C	18°C		20°C		20°C	18°C	

KEY.

Effect of oestradiol benzoate on normal females. (Cont.)

	39 Hours		48 Hours		60 Hours		72 Hours		96 Hours.	
	Oest.	Cont.	Oest.	Cont.	Oest.	Cont.	Oest.	Cont.	Oest.	Cont.
	4.5	5.5	3.4	5.1	4.3	5.0	4.0	4.5	4.5	5.9
	4.8	5.5	3.3	4.8	4.5	4.8	3.8	5.4	4.7	5.5
	4.9	4.3	3.0	5.5	3.1	4.5	3.9	5.3	4.5	5.3
	4.4		3.5	5.0		5.0	3.3	5.3	4.0	
			2.9	5.1					4.5	
AVERAGE	4.7	5.1	3.2	5.1	4.0	4.8	3.8	5.1	4.4	5.5
No. of toads	7	7	7	7	6	7	7	7	8	8
Temperature	18°C		20°C		20°C		18°C		20°C	

XXV.

PROTOCOL NO. 18.

Effect of repeated doses of progesterone and oestradiol benzoate.

	24 Hours			48 Hours		
	Progest.	Oestrad.	Control	Progest.	Oestrad.	Control
	5.6	4.2	4.6	4.4	4.0	4.6
	5.4	4.1	4.8	4.1	3.7	4.5
	5.4	5.6	4.3	4.8	3.8	4.5
	4.8	5.6	4.8	4.5	4.2	5.3
	4.9	5.8	4.8	4.6		4.8
			4.9		(5.5)	
AVERAGE	5.2	5.1	4.7	4.5	3.9	4.7
No. of toads	8	7	8	7	7	7
Temperature		22°C			22°C	

XXXI.

PROTOCOL NO. 19.

Effect of thyroidectomy.

	Normal	1 Month Thyroid.Op.Cont.		2 Months Thyroid.Op.Cont.		3 Months Thyroid.Op.Cont.	
	4.0	4.6	4.3	6.1		4.9	4.5
	4.3	4.5	5.5	6.0	4.9	4.8	5.6
	5.0	5.0	5.7	5.0	4.9	4.9	5.0
	4.8			4.5	3.8	5.2	5.0
	4.9			4.6	4.2	5.3	5.2
	5.0			5.2	4.5	5.5	
AVERAGE	4.7	4.7	5.2	5.2	4.5	5.1	5.1
No. of toads	12	7	7	8	8	9	8
Temperature	19.5°C			15°C		17°C	

XXVII.

Effect of thyroidectomy (cont.)

5 months		6 Months		7 Months	
Thyroid.	Capt. Cont.	Thyroid.	Capt. Cont.	Thyroid.	Capt. Cont.
4.6	5.4	4.1	5.0	5.1	4.4
4.5	5.8	4.3	5.1	4.4	4.7
5.5	4.5	5.4	5.8	5.2	5.3
5.4	4.8	5.7	6.1	5.3	5.6
4.8	5.6	5.1	5.9		
5.0		5.3			
5.2		5.4			
5.0					
AVERAGE 5.0	5.3	5.0	5.8	5.0	5.0
No. of toads 7	9	9	10	7	10
Temperature	19.5°C		21°C		22.5°C

XXVIII.

PROTOCOL NO. 20.

Effect of thyroxine on normal females.

	Normal	12 Hours Thyr.	12 Hours Cont.	24 Hours Thyr.	24 Hours Cont.	36 Hours Thyr.	36 Hours Cont.	48 Hours Thyr.	48 Hours Cont.	60 Hours Thyr.	60 Hours Cont.	72 Hours Thyr	72 Hours Cont.
	5.4	4.7	4.9	3.9	5.2	3.9	5.2	4.4	5.2	4.8	4.8	5.5	4.5
	5.0	4.4	4.7	4.3	4.9	4.2	4.4	3.8	4.6	5.2	4.6	4.2	4.8
	4.0	4.2	4.9	3.7	4.8	(5.7)	4.8	4.0	4.6	4.8	4.8	4.0	5.5
	5.0	4.1	5.3	4.0	4.8		4.9	5.0	4.9	5.2		5.1	5.5
	5.2	4.7	5.3		5.3		4.9					4.8	
				4.2	5.1								
					4.9								
AVERAGE	4.9	4.4	5.0	4.0	5.0	4.6	4.8	4.3	4.8	5.0	4.7	4.7	5.1
No of toads	11	7	7	7	7	7	7	7	7	7	7	7	7
Temperature	19°C	19°C		19°C		18°C		19°C		19°C		20°C	

PROTOCOL NO. 21.

Effect of repeated injections of thyroxine.

	Normal	24 Hours		48 Hours	
		Thyroxine	Control	Thyroxine	Control
	5.4	6.0	4.3	5.4	4.5
	5.0	6.6	5.0	6.3	5.4
	4.0	6.5	4.7	6.7	5.6
	5.0	5.4	5.5	5.8	
	5.2	5.6		5.7	
				6.7	
AVERAGE	4.0	6.0	4.9	6.1	5.2
No. of toads	11	8	8	9	9
Temperature	19°C	21°C		21°C	

	24 Hours		48 Hours	
	Thyroxine	Control	Thyroxine	Control
	4.6	4.6	5.4	4.6
	5.0	4.8	5.1	4.5
	5.4	4.3	5.6	4.5
	5.6	4.8	4.9	5.3
		4.8	5.8	4.8
		4.9		
AVERAGE	5.2	4.7	5.4	4.7
No. of toads	8	8	7	7
Temperature	22°C		22°C	

PROTOCOL NO. 22.

Effect of Pancreatectomy on plasma inorganic phosphate and blood sugar.

Normal	3 Hours		6 Hours		12 Hours		24 Hours		48 Hours	
	Panc.	Op. Con.	Panc.	Op. Con.	Panc.	Op. Con.	Panc.	Op. Con.	Pan.	Op. Con.
6.3	8.6	7.1	9.4	6.3	7.9	6.2	7.3	6.0	6.5	5.9
6.1	7.8	6.4	7.7	6.8	7.1	6.1	7.5	4.9	7.3	6.0
6.1	8.0	6.4	8.0	7.2	9.9	6.9	6.2		6.1	5.1
6.5	6.9		8.7	6.9	7.2	6.9	6.0		6.5	
6.7	8.9		7.7	7.3	8.5		7.8			
	7.8		7.6		9.2		6.0			
			8.1		8.1		6.7			
					8.0		6.5			
AVERAGE 6.3	8.0.	6.6	8.2	6.9	8.2	6.5	6.8	5.5	6.6	5.7

BLOOD SUGAR.

Normal	6 Hours Panc.Op.Con.		12 Hours Panc.Op.Cont.		24 Hours Panc.Op.Cont.		48 Hours Panc.Op.Con.	
37	115	70	115	92	92	12	48	49
48	138	70	126	60	48	18	60	49
48	160	92	92	37	70	24		12
48	126	60	149	37	82	24		
37	104	70	126		48			
	115	60			92			
	160				70			
					104			
AVERAGE 44	131	70	122	37	76	20	54	37
Temperature 22°C	19.5°C		21.5°C		20°C		19°C	

PROTOCOL NO. 24.

Effect of insulin on plasma inorganic P and Blood Sugar.

Normal P.	Sugar	1 Hour P.	Sugar	2 Hours P.	Sugar	3 Hours P.	Sugar	4 Hours P.	Sugar	6 Hours P.	Sugar	12 Hours P.	Sugar
6.1	78	4.0	65	4.9	37	4.0		3.5	25	6.0		6.0	
6.0		6.5	48	4.7	48	4.1		3.5	30	5.9		5.7	
7.4		5.8		3.8		3.9		2.9		5.3		5.7	
7.9		5.6		4.7		4.0		3.2		5.5		5.5	
6.6		5.7		4.5		3.6				5.7			
6.8		6.0											
6.6													
7.4													
7.1													
6.3													
7.2													
AVERAGE 6.9	78	5.6	56	4.5	42	3.9		3.3	27	5.7		5.7	
Temperature	21°C		21°C		21°C		21°C		21°C				

XXIII.

Effect of ether

	Ether	Control
	6.0	6.0
	8.5	8.7
	7.7	7.9
	8.3	9.0
	6.0	6.9
AVERAGE	7.7	7.7

PROTOCOL NO. 25.

	Normal	3 Hours Ins.	3 Hours Cont.	6 Hours Ins.	6 Hours Cont.	12 Hours Ins.	12 Hours Cont.	24 Hours Ins.	24 Hours Cont.	3 Hours Ins.	3 Hours Cont.	12 Hours Insulin	48 Hrs. Ins.	48 Hrs. Cont
	5.2	6.0	5.7	4.2	6.3	3.6	6.7	4.2	5.4	5.4	6.9	5.5	7.9	5.8
	5.6	4.4	5.7	5.0	5.6	3.6	6.4	4.5	5.5	4.1	6.9	4.9	6.8	5.9
	6.0	4.5	5.7	4.3	6.5	4.9	6.6	5.3	5.1	4.5	5.5	4.7		
		5.0		4.5	6.2	5.0		5.6	5.3	5.9	6.0	4.8		
								5.9	5.4					
AVERAGE	5.6	5.0	5.7	4.5	6.2	4.3	6.6	5.1	5.3	5.0	6.2	5.0	7.4	5.9

PROTOCOL NO. 26.

Effect of insulin on normal females.

	Normal	6 Hours		24 Hours	
		Insulin	Control	Insulin	Control
	6.3	4.9	5.8	4.4	5.6
	6.5	5.0	5.4	5.2	5.9
	6.8	4.8	5.8	5.7	6.2
	7.1	4.6	6.4	5.3	
		4.7			
		5.1			
		5.2			
		5.1			
AVERAGE	6.7	4.9	5.8	5.2	5.9

	Normal	24 Hours		40 Hours		50 Hours	
		Insulin	Control	Insulin	Control	Insulin	Control
	6.9	5.0	6.6	5.6	6.9	4.8	6.0
	7.3	4.8	6.5	5.0	6.3	4.6	6.3
	6.7	5.0	5.9	4.6	6.6	5.8	5.6
		4.6	6.5	5.4	6.2	(7.3)	
		4.5		5.4			
AVERAGE	6.9	4.8	6.4	5.2	6.5	5.1	6.0
No. of toads	10	7	8	7	8	9	11
Temperature	22°C	23°C		22.5°C		22.5°C	

PROTOCOL NO. 26.
(cont.)

	Normal	50 Hours		64 Hours		74 Hours	
		Insulin	Control	Insulin	Control	Insulin	Control
	5.4	4.3	5.5	5.5	5.9	4.6	5.7
	5.2	6.8	4.9	7.5	5.3	7.9	5.8
	5.3	8.2	5.0		5.2		6.0
	5.1	6.4			5.4		
	5.0						
AVERAGE	5.2	6.4	5.1	6.5	5.4	6.3	5.8
No. of toads	12	7	9	5	9	6	8

SEMI COMATOSE

PROTOCOL NO. 23.

Effect of insulin in pancreatectomised animals.

	Normal	Pancreatectomy	4 Hours Insul.Cont.	6 Hours Insul.Cont.	18 Hours Insul.Cont.
	4.8	7.8	5.8 6.1	5.8 6.4	5.4 5.9
	5.5	6.0	5.8	5.6 5.9	5.3 6.0
	5.0				
AVERAGE	5.1	6.9	5.8 6.1	5.7 6.2	5.4 6.0
No. of toads	10	12	8 8	7 7	6 6
Temperature			14.5°C		

Effect of S.A.P.1B in pancreatectomised animals.

	Normal	Pancrea-tectomy	3 Hours S.A.P.1B	3 Hours Control	6 Hours S.A.P.1B	6 Hours Control	12 Hours S.A.P.1B	12 Hours Control
	4.8	7.8	6.4	6.1	6.3	6.4	(5.3)	5.9
	5.5	6.0	6.6		6.0	5.9	6.0	6.0
	5.0						6.6	
AVERAGE	5.1	6.9	6.5	6.1	6.1	6.2	6.0	6.0
No. of toads	10	12	9	8	9	7	7	6

Temperature 14.5°C

Pancreatectomised toads	Insulin	52	survivals	21	
"	"	Ringer	52	"	21
"	"	S.A.P.1B	52	"	25

xxxix.

PROTOCOL NO. 27.

Effect of adrenaline on normal females.

	Normal	1.5 Houre Adren.	Control	3 Hours Adren.	Control	24 Hours Adren.	Control
	7.4	(8.7)	7.9	5.4	8.7	6.5	6.5
	7.5	6.3	8.1	5.3	8.1	6.9	7.0
	7.8	5.9	7.6	5.6	8.1		6.9
				5.5	8.2		
AVERAGE	7.6	7.0	7.9	5.5	8.3	6.7	6.8

	Normal	3 Houre Adren.	Cont.	6 Hours Adren.	Cont.	10 Houre Adren.	Cont.	24 Houre Adren.	Cont.
	7.0	6.2	6.6	6.2	7.3	6.5	6.2	6.7	5.8
	6.8	6.1	6.7	6.5	7.5	7.6	6.2	6.7	6.4
	6.8	6.1	6.9	5.8	6.4	7.5	6.8	6.7	7.2
	6.8	5.9	7.2	6.4	6.8	7.6	6.5	6.1	
			7.3	6.0	7.2		6.7		
			7.2		7.0				
AVERAGE	6.8	6.1	7.0	6.2	7.0	7.3	6.5	6.6	6.5

PROTOCOL NO. 27
(cont.)

Effect of adrenaline on normal females.

	Normal	2 Hours Adren.	Cont.	4 Hours Adren.	Cont.	6 Hours Adren.	Cont.	9 Hours Adren.	Cont.	14 Hours Adren.	Cont.
	5.3	4.6	5.8	4.9	5.5	4.8	5.7	4.9	5.6	6.2	6.4
	5.6	5.2	6.2	5.0	6.0	4.4	6.5	5.2	5.3	5.6	5.5
	6.0		5.9	4.6	5.5		6.4			5.9	6.1
	6.6		6.4	5.1	6.4						6.0
	6.3										
AVERAGE	6.0	4.9	6.1	4.9	5.9	4.6	6.2	5.1	5.5	5.9	6.0
No. of toads	8	7	7	7	7	7	6	6	8	8	7

PROTOCOL NO. 27 (cont.)

	18 Hours		24 Hours		36 Hours	
	Adren.	Control	Adren.	Control	Adren.	Control
	6.1	5.9	7.2	5.6	5.8	5.8
	5.5	6.4	7.9	5.1	6.6	6.3
	6.0			6.2	6.2	6.3
	6.5				6.6	
AVERAGE	6.0	6.1	7.5	5.6	6.3	6.1
No. of toads	8	8	7	8	8	9
Temperature			16°C			

	Normal	12 Hours	
		Adrenaline	Control
	4.4	5.8	3.2
	5.1	6.1	4.1
	3.9	6.2	4.4
	3.9	6.2	3.6
	4.7	6.6	4.2
	4.1		4.8
AVERAGE	4.4	6.2	4.1
No. of toads	15	12	12
Temperature		18.5°C	

PROTOCOL NO. 28.

Effect of insulin on hypophysectomised animals.

	Normal	2 Month Hypoph.	4 Hours Ins.	Cont.	8 Hours Ins.	Cont.	12 Hours Ins.	Cont.	19.5 Hours Ins.	Cont.	25 Hours Ins.	Cont.
	5.0	4.4	2.9	3.8	3.5	4.4	4.0	3.9	3.3	4.5	3.4	4.3
	5.6	4.3	3.0	4.1	3.2	3.9	4.0	4.4	3.4	4.3	4.4	4.8
	5.6	4.6	3.2	3.7	3.7	4.4	4.1	4.7	4.1	4.7	4.6	5.1
	4.9	4.8	3.6	3.9	3.9	4.4	3.5		3.8		4.0	5.1
	5.0						3.7		3.9		3.6	
									4.0		3.4	
AVERAGE	5.2	4.5	3.2	3.9	3.6	4.3	3.9	4.3	3.8	4.5	3.9	4.8
No. of toads	9	11	8	7	8	7	8	7	8	5	8	6

Temperature 15°C

PROTOCOL NO. 28
(cont.)

37.5 Hours Insulin	Contr.	50 Hours Insulin	Contr.
4.4	4.0	4.0	4.6
3.7	4.4	4.3	4.8
3.8		4.0	
4.0		4.4	
4.2			
AVERAGE 4.0	4.2	4.2	4.7
No. of toads 8	4	9	6

Effect of insulin on operative control animals.

Normal	12 Month Op.	Cont.	4 Hours Ins.	Cont.	8 Hours Ins.	Cont.	12 Hours Ins.	Cont.	19.5 Hours Ins.	Cont.	26 Hours Ins.	Cont.
5.0	5.1	3.9	4.7	3.9	4.8	3.9	5.2	3.9		4.0	5.2	
5.6	5.2	3.4	4.6	4.0	5.7	4.2	6.3	3.0	5.0	4.1	5.9	
5.6	5.3	3.6	5.1	4.2	5.3	3.9	5.4	3.3	5.5	4.4	5.7	
4.9	5.5	3.8		4.0		3.8		3.3		4.1		
5.0	5.6			4.1		4.1		3.4		4.2		
								3.8		3.9		
								3.4		4.5		
AVERAGE 5.2	5.3	3.7	4.8	4.0	5.3	4.0	5.6	3.4	5.3	4.2	5.6	
No. of toads 9	11	8	8	9	7	8	7	8	7	8	7	
Temperature			15°C									

PROTOCOL NO. 26.
(cont.)

	37.5 Hours		50 Hours	
	Insulin	Control	Insulin	Control
	5.0	5.5	4.9	5.0
	4.0	5.9	5.1	4.8
	4.0	6.5	5.5	5.5
	4.4		4.4	6.0
	4.0		4.9	
	4.6			
AVERAGE	4.3	5.9	5.0	5.3
No. of toads	8	7	8	8

Effect of insulin on hypophysectomised animals (continued).

6 week hypophysectomy

Pre-injection	2 hours after insulin
5.8	5.8
4.5	4.9
4.3	5.2
	5.4
AVERAGE 4.9	5.2

PROTOCOL NO. 29.

Effect of adrenaline on hypophysectomised animals

	Normal	2month hypoph.	2Hours	3Hours	4Hours	5Hours	12Hours	19Hours	25Hours	36Hours.
	5.0	4.4	4.6	5.1	4.7	4.5	4.8	5.4	5.3	6.0
	5.6	4.3	4.7	4.8	4.5	4.9	4.8	5.5	5.5	6.2
	4.9	4.6	5.1	4.7	4.8		4.3	5.9	5.5	
	5.0	4.8	5.1							
	5.6	4.5								
		4.4								
AVERAGE	5.2	4.5	4.9	4.9	4.7	4.7	4.6	5.6	5.4	6.1
No. of toads	9	18	9	9	10	9	8	9	9	8

PROTOCOL NO. 29.
(cont.)

Effect of adrenaline on operative control animals.

	Normal	2 month Op. Cont	2 Hours	3 Hours	4 Hours	5 Hours	6 Hours	12 Hours	19 Hours	25 Hours	36 Hours.
	5.0	5.1	5.8	4.8	4.8	5.2	4.8	5.4	6.2	6.5	6.5
	5.6	5.2	5.1	4.5	4.7	4.7	4.5	5.7	6.4	6.8	6.7
	5.5	5.3	5.5				4.9		6.0	7.0	
	4.9	5.5							6.3		
	5.0	5.6									
AVERAGE	5.2	5.3	5.5	4.7	4.7	5.0	4.7	5.5	6.2	6.8	6.6
No. of toads	9	11	12	12	12	13	9	8	10	9	10

XVII.

PROTOCOL NO. 29.
(cont.)

	2 month hypophysec	adrenaline 3 Hours	2 month Op.Control	Adrenaline 3 Hours
	4.4	4.9	5.1	4.0
	4.3		5.6	
	4.6	4.3	5.5	3.9
	4.8		5.3	
	4.5		5.2	
	4.4			
AVERAGE	4.5	4.6	5.3	4.0
No. of toads	18	8	11	8

PROTOCOL. NO. 30.

Effect of S.A.P.2B. on ovariectomised animals.

Normal	2 Month Ovari.	6 Hours S.A.P.	Cont.	12 Hours S.A.P.	Cont.	18 Hrs. S.A.P.	26 Hours S.A.P.	Cont.	36 Hrs. S.A.P.	48 Hours S.A.P.	Cont.
4.6	5.0	5.4	4.7	3.9	5.6	4.0	4.6	4.5	4.0	4.3	5.9
4.8	4.8	5.4	5.9	4.5	5.4	4.5	5.1	4.9	3.7	4.9	6.0
4.3	4.9	5.4	5.5	5.4	5.9	4.7	4.9	5.2	3.8	3.4	5.4
4.8	5.2	5.5	5.4	6.0	5.5	5.2	5.4	5.7	4.1	3.4	4.8
4.8	5.8	5.5	5.6	4.2	5.6	4.7	5.6	5.8	4.0	2.8	4.5
4.9	5.2	6.2	5.8	4.8		4.6	5.2	5.8	3.4	(2.0)	4.9
4.6	4.9	6.5				5.0	4.7	5.8	4.3		5.6
4.5	5.4						4.3		4.0		5.7
4.5	6.2								4.3		
5.3	5.9										
4.8											
AVERAGE 4.7	5.3	5.7	5.5	4.8	5.6	4.7	5.0	5.4	4.0	3.8	5.4
No. of toads 15	10	7	7	8	7	7	7	6	6	6	9
Temperature 22° C	18° C	18° C		19.5° C		19.5° C	19.5° C		19.5° C	18.5° C	

PROTOCOL NO. 30.
(cont.)

Effect of S.A.P.2B on operative control animals.

Normal	2 Month Op.Cont	6 Hours S.A.P.	Cont.	12 Hours S.A.P.	Cont.	18 H S.A.P.	26 Hrs. S.A.P.	Cont.	36Hrs. S.A.P.	48 Hours S.A.P.	Cont.
4.6	5.4	5.6	5.6	6.0	4.9	4.0	4.6	5.3	3.6	3.3	5.0
4.8	5.0	5.5	4.5	5.6	5.6	4.5	5.0	5.0	3.9	2.9	5.1
4.3	5.4	5.4	5.0	6.3	5.5	4.7	5.9	5.9	4.1	3.7	5.5
4.8	4.4	5.8	5.6	5.6	5.3	5.0		5.7	3.8	3.3	5.2
4.8	5.1	5.7	5.7	6.2		4.3		5.0			5.8
4.9	4.7			6.6		4.8		5.3			
4.6	6.1										
4.5	6.1										
4.5											
5.3											
4.8											
AVERAGE 4.7	5.3	5.6	5.3	6.1	5.3	4.6	5.2	5.4	3.9	3.3	5.3
No.of toads 15	10	7	6	8	7	7	7	6	6	6	9
Temp. 22°C	18°C	18°C		19.5°C		19.5°C	19.5°C		19.5°C	18.5°C	

PROTOCOL NO. 31.

Effect of progesterone and oestradiol benz. on hypophysectomised toads.

	Normal	2 Months Hypophys.	12 Hours Prog.	12 Hours Oest.	12 Hours Cont.	24 Hours Prog.	24 Hours Oest.	24 Hours Cont.	36 Hours Prog.	36 Hours Oestr.	36 Hours Control.
	5.4	4.4	4.0	4.1	4.4	3.0	3.9	4.3	3.4	4.1	4.0
	5.0	4.5	4.1	4.0	4.7	3.0	3.5	4.5	3.5	4.2	4.4
	4.0	4.6	4.3	3.1	5.0	3.1	3.5	4.7	2.4	3.5	4.7
	5.0		3.9	3.8	5.0		2.7		3.9	4.0	4.4
	5.2			3.7			4.0			4.0	
				3.4							
AVERAGE	4.9	4.5	4.1	3.7	4.8	3.0	3.5	4.5	3.3	4.0	4.4
No. of toads	11	9	7	7	6	8	7	5	7	7	6
Temperature	19°C	14.5°C	18°C	15°C			18°C			18°C	
Ov. Count			0,0,0 0,0 0,0			0,1,1 0,1,0 0,0			0,0,0 0,0 0,0		

Tab.

PROTOCOL NO. 31.

	48 Hours			72 Hours		96 Hours	
	Prog.	Oest.	Cont.	Prog.	Oestr.	Prog.	Oestr.
	3.9	3.3	4.5	3.9	4.3	3.3	4.5
	3.2	3.2	4.4	3.6	3.6	3.5	3.1
	4.1	3.6		4.1	3.4	3.5	
	4.0	3.1		4.1	3.6	3.8	
				4.6		3.3	
				4.2			
AVERAGE	3.8	3.3	4.5	4.1	3.7	3.5	3.8
No. of toads	6	7	6	8	7	7	7
Temperature		18°C			18°C		18°C
Ov. Count	0,4 4,0 0,0			0,0 1,0,3 0,0,0		4,0 0,0 0,0,4	

PROTOCOL NO. 31.
(cont.)

Effect of progesterone and oestradiol benz. on operative controls.

	Normal	2 Month Hypophy.	12 Hours Prog.	12 Hours Oest.		12 Hours Cont.	24 Hours Prog.	24 Hours Oest.	24 Hours Cont.	36 Hours Prog.	36 Hours Oest.	36 Hours Cont.
	5.4	5.2	5.0	4.3	ESCAPED		4.5	4.3	5.4	4.2	4.0	6.0
	5.0	5.5	5.1	4.1			4.6	4.6	5.3	3.2	3.9	5.5
	5.0	5.5	5.0	4.6			4.1	3.1		3.8	3.6	6.3
	4.0	5.6	4.7	4.4			3.0	3.5		3.7	4.0	
	5.2											
AVERAGE	4.9	5.5	5.0	4.4			4.1	3.9	5.0	3.7	3.9	5.9
No. of toads	11	9	7	6			7	7	6	6	6	6
Temperature	19°C	14.5°C	18°C				18°C			18°C		15°C
Ov. Count			0,0 1,0 0,0,0				0,0 0,4 1,0,0			0,0 0,0 0,0		

PROTOCOL NO. 31
(cont.)

	48 Hours		72 Hours		96 Hours
	Progest.	Oestrad.	Progest.	Oestrad.	Progesterone.
	4.3	4.3	4.1	5.6	4.5
	4.3	4.5	4.4	5.7	4.3
	4.6	3.9	3.8	4.3	3.2
	4.5	4.5	4.1		4.4
	4.1				
AVERAGE	4.4	4.3	4.1	5.2	4.1
No. of toads	7	7	7	6	7
Temperature	18°C		18°C		18°C
Ov. Count	5,6 6,6 6,0,5		4,4 4,4 0,0,4		5,5 0,2 4,0,4

PROTOCOL. NO. 32.

Effect of parathyroid hormone on hypophysectomised animals.

	Normal	5 Month Hypoph.	Hours 6	Hours 12	Hours 18	Hours 24	Hours 48	Hours 60	Hours 72
	4.8	5.2	3.5	3.7	3.9	5.0	5.3	5.8	6.1
	4.4	5.0	3.3	3.9	2.7	5.4	5.2	5.7	6.0
	4.6	4.5	3.3	5.1	2.9	5.5	6.0	6.1	5.7
	4.5	4.4	3.9	3.1	3.4	5.3	6.0	5.1	5.4
	4.8	4.9		3.6	3.6	6.0	6.1	6.2	6.2
	5.5	5.2		3.2		6.1	5.5	6.4	6.1
	4.9	4.7				5.4	5.7		
	5.2	4.8							
		5.1							
AVERAGE	4.8	4.9	3.4	3.8	3.3	5.5	5.7	5.9	5.9
No. of toads	12	18	7	7	7	7	7	7	8
Temperature	21°C	16.5°C	16°C	16.5°C	16°C	16.5°C	17°C	17°C	17°C

PROTOCOL NO. 32. (cont.)

Effect of parathyroid hormone on operative control animals.

	Normal	5 Month Hypoph.	Hours 6	Hours 12	Hours 18	Hours 24	Hours 48	Hours 60	Hours 72
	4.8	5.2	4.2	4.1	3.6	5.6	5.0	5.2	5.4
	4.4	5.1	4.6	4.5	3.3	6.0	5.4	6.1	5.7
	4.6	5.7	5.1	5.3	3.3	6.2	5.7	7.3	6.3
	4.5	6.0			4.0	6.2	5.8	6.8	6.5
	4.8								
	5.5								
	4.9								
	5.2								
AVERAGE	4.8	5.5	4.6	4.6	3.6	6.0	5.5	6.4	6.0
No. of toads	12	11	7	7	7	8	7	7	8
Temperature	21° C	16.5° C	16° C	16.5° C	16° C	16.5° C	17° C	17° C	17° C

PROTOCOL NO. 33.

Effect of thyroxine on hypophysectomized animals.

	Normal	5 Month Hypoph.	Hours 24	Hours 48	Hours 72	Hours 96	Hours 144
	4.8	5.2	3.4	2.8	4.4	4.9	5.5
	4.4	5.0	3.9	3.9	5.5	5.0	4.4
	4.6	4.5	3.5	3.4	6.0	5.4	4.5
	4.5	4.4	4.7	3.6	4.8	5.7	4.8
	4.8	4.9	4.4			4.6	
	5.5	5.2	4.5			5.0	
	4.9	4.7					
	5.2	4.8					
		5.1					
AVERAGE	4.8	4.9	4.1	3.4	5.1	5.1	4.8
No. of toads	12	18	7	7	7	7	7
Temperature	21°C	16.5°C	16°C	10°C	16.5°C	17°C	17.5°C

PROTOCOL NO. 33.
(Cont.)

Effect of thyroxine on operative control animals.

	Normal	5 Month Op. Cont.	Hours 24	Hours 48	Hours 96	Hours 120	Hours 144
	4.8	5.2	4.9	2.7	5.5	5.6	5.4
	4.4	5.1	4.9	3.9	6.0	6.3	6.2
	4.6	5.7	5.2	3.8	5.5	5.6	
	4.5	6.0		4.1	6.2		
	4.8						
	5.5						
	4.9						
	5.2						
AVERAGE	4.8	5.5	5.0	3.6	5.8	5.8	5.8
No. of toads	12	11	7	7	8	8	8
Temperature	21°C	16.5°C	16°C	18°C	17°C	17°C	17.5°C