STUDIES ON

RENAL FUNCTION DURING PROFOUND HYPOTHERMIA

in the dog.

A Thesis submitted to the University of Cape Town for the degree of Doctor of Medicine.

by

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To

MY FATHER.

"What matters to me, is not merely to impart to the reader what I have to say, but above all to convey to him the reasons, subterfuges and lucky hazards which led me to my discoveries. When Christopher Columbus, Magelhaen and the Portuguese relate how they went astray on their journeys, we not only forgive them, but would regret to miss their narration, because without it the whole grand entertainment would be lost. Hence I shall not be blamed if, prompted by the same affection for the reader, I follow the same method".

Johannes Kepler, 1609.

CONTENTS.

ABBREVIATIONS.

NOTE: (1) Throughout this work, the temperature is given in degrees Centrigrade, unless otherwise stated.

(2) "Profound Hypothermia" is surgical jargon for a body temperature of less than 20°C.

ADH	:	anti-diuretic hormone
Ca	:	calcium
Ccr	:	creatinine clearance
C_{IN}	:	inulin clearance
C_{PAH}	:	Para-aminohippurate clearance
ECG	:	Electrocardiogram
ECF	:	extracellular fluid
ERPF	:	effective renal plasma flow
$\Delta F^{\ddagger}$	:	change in Free Energy of Activation
GFR	:	glomerular filtration rate
$\Delta H^{\ddagger}$	:	change in Heat Content, or Enthalpy, of Activation
K	:	potassium
Mg	:	magnesium
mOsm/l.	:	milli-osmoles per litre
Na	:	sodium
P	:	inorganic phosphate
PAH	:	para-aminohippurate
P_{osm}	:	plasma osmolality
RBF	:	renal blood flow
$\Delta S^{\ddagger}$	:	change in Entropy of Activation
Tm	:	maximal rate of tubular transport
U_{osm}	:	urinary osmolality

PREFACE.

During the academic year 1960-1961, the Department of Experimental Surgery, of the University of Cape Town, engaged in a series of experiments concerning the production of profound hypothermia in dogs. Hypothermia was induced by blood-stream cooling, via a heat exchanger incorporated in an extra-corporeal pump-oxygenator system (cf Appendix A). Progress was such that by midyear, postoperative survival of experimental animals was assured.

At this juncture, it was suggested that, as a member of the Renal-Metabolic Group of the Department of Medicine, I co-operate with the surgeons, in a study of renal function during profound hypothermia in the dog.

This Thesis details our progress and results.

CHAPTER 1.

HISTORICAL REVIEW.

Interest in the renal effects of hypothermia may be said to date back more than one hundred years, when Claude Bernard (16) first noted the presence of glycosuria in animals cooled to 20°C following immersion in ice water.

In 1880, Lassar (98) using the same technique induced mild hypothermia (32°C) in rabbits. He found that albuminuria and hyaline cylindruria occurred consistently, and often persisted for days. Subsequent microscopic examination of the kidneys revealed inflammatory interstitial changes.

Albuminuria, as a concomitant to hypothermia, was noted many times thereafter, both in animals (62, 113, 186) and humans (143). Opinion as to the presence or otherwise of cold-induced microscopic renal abnormality, differed. Thus Zillessen (186) described hyperaemic glomeruli, areas of hyaline degeneration, cortical haemorrhages, and hyaline and red blood cell casts in the tubules; while Giese (62) on the other hand, could find no abnormality in the kidneys of hypothermic albuminuric rabbits.

Hallion and Ambard (70) noted a decreased urinary urea concentration, and a greater rate of secretion of urine, in hypothermic dogs. Conway (36), working with rabbits, found the rate of urine secretion to be variable, but usually to rise with initial cooling, only to fall later, and this with no drop in blood pressure. He also noted a sharp fall in urinary chloride concentration.

Schlomka (143) attempted to cool the kidneys by a rather novel method and one which might be thought of as a primitive precursor to the extracorporeal cooling techniques in vogue today. Using human subjects, he immersed the legs, only, in ice water for about 15 minutes, while a loosely applied tourniquet occluded the venous return. On releasing the tourniquet, cold blood ascended to the trunk and presumably soon reached the kidneys. About three quarters of his 37 human subjects then developed an acute fall in urine flow rate, accompanied by a marked rise in urinary specific gravity, and albuminuria.

The twenties and early nineteen thirties brought about a revolution in thinking on renal mechanics. The work of men such as A.R. Cushny, A.N. Richards and H.W. Smith ushered in an era of direct renal experimentation, and of new concepts which still govern our ideas of renal

physiology today. 1937 saw the publication of a new classic paper by Bickford and Winton (18) on "The influence of temperature on the isolated kidney of the dog". This was the first paper on the subject to utilise the new concepts, and was to remain unchallenged for almost twenty years. Their experimental model was the isolated canine kidney, perfused with defibrinated blood. By varying the temperature of the perfusing blood, kidney temperatures could be brought to as low as 3°C. Their results given here in abbreviated form, will be referred to again in later chapters:

(a) Cooling the kidney to 13° to 3°C, changes the urinary composition substantially to that of a serum transudate.

(b) Kidney function, as evaluated by urinary composition and the rate of renal blood flow, recovers completely, and promptly, on rewarming the cold organ.

(c) The urine flow rate increases with cooling, to reach a maximum at about 10°C.

(d) The minimum arterial pressure needed to produce urine, in the warm kidney uninfluenced by diuretics, is 70-80 mm Hg. In the cold kidney, this figure falls to 40-50 mm Hg.

(e) Cooling reduces renal blood flow in approximately the same proportion in the isolated kidney as in a glass viscosimeter i.e. the fall in RBF is due largely, if not entirely, to the raised viscosity of cold blood.

(f) At body temperature, urinary chloride concentration in the isolated kidney is always low. This rises on cooling to reach levels equal to that of serum, at about 18°C.

(g) The creatinine clearance is markedly reduced by cooling.

During the forties, prolonged moderate hypothermia was used, experimentally, in the treatment of schizophrenia. Talbott and others,(169) seized this opportunity to study renal function in man during hypothermia. An early report in 1941 concerned a patient who had succumbed during this therapy. The patient's rectal temperature had been lowered by means of a refrigerating blanket to 80°F (27°C), and kept at that level for 48 hours, before death supervened. The urine remained albumen and sugar free throughout, with increased concentrations of phosphate and nitrogenous

products. At autopsy, no abnormality could be detected in the kidneys. By 1952 Talbott (170) was able to report on a further 15 cases, all mental patients in a psychiatric hospital, who had been subjected to hypothermia for 2 or more days. Brine cooled blankets were used to attain rectal temperatures of 80°F (27°C), and in one case of 74°F (23.3°C). The GFR and RBF were found to fall linearly with a falling rectal temperature, so that by 85°F (29.4°C) the GFR had fallen 50%. Albuminuria was noted in only a few cases. No striking change occurred in urine flow rate. The Tm diodrast fell even more sharply than the GFR. Repeated studies, after rewarming, revealed no abnormality in GFR, Tm diodrast or C diodrast.

Further evidence for the rapid reversal, and non-pathogenicity, of the paralysis of renal function produced by cold, was adduced by Forster and Taggart in 1950 (58). Working with isolated renal tubules derived from freshly caught winter flounders (a variety of marine teleost, and one of several which possess the experimentally desirable quality of having very little renal intertubular cement substance) they were able to show that chilling produced a profound paralysis of phenol red transport. Rewarming of the tubules, even after several hours of chilling, led to immediate and complete resumption of phenol red transport.

Three years later, and now using the intact longhorn sculpin (a glomerular teleost); Forster (59) showed that a similar cold sensitive transport mechanism existed for PAH.

The early nineteen fifties witnessed a renaissance in cardiac surgery. Emboldened by initial successes, the surgeons soon began to cast about for a means of obtaining a bloodless intracardiac field. The obvious solution was the use of an extracorporeal blood pumping device, to maintain a continuous flow of blood while the heart was diverted from the circulation. Such machines had, in fact, been in existence, and experimental use, since 1939 (61). The problem of supplying an adequate rate of (oxygenated) blood flow for total body perfusion remained a formidable one, however, and was never really solved. Accordingly, it was soon suggested, as an alternative to cardiac bypass, that hypothermia be used to reduce tissue metabolism and oxygen requirements, so that susceptible tissues would be able to survive prolonged periods of ischaemia without irreversible damage (20). Initially, hypothermia was induced by surface cooling techniques; later by cooling the blood during its course through a man-made extracorporeal arterio-venous fistula (25, 44). It was not long before the two methods

were combined, viz. hypothermia together with an extra-corporeal circulation (63, 66). This latter technique proved to be the solution the surgeons were waiting for; as Gollan wrote (67): "only by making the circulation and oxygenation of blood independent of the haemodynamic changes of induced hypothermia has the barrier to the rate, degree and duration of cooling of large animals been removed". It had now become possible to induce complete cardiac arrest for periods of up to one hour, with subsequent survival (64).

This activity by the cardiologists proved a powerful stimulus to other workers in the field of hypothermia.

More papers (2, 3, 4, 13, 17, 22, 41, 74, 77, 78, 80, 81, 86, 88, 114 to 117, 119, 127, 138, 146, 147, 166) on the renal effects of hypothermia appeared during the years 1953-61 than in the whole of the preceding 100 years. The favourite experimental animal has been the dog, but rabbits (2), alligators and turtles (77), squirrels (78), rats (3, 4, 78), bullfrogs (81), and lambs (115) have also been used. Few reports (17, 116, 119) have dealt with man, and these never at temperatures lower than 27°C. Most studies on intact dogs were

performed at rectal temperatures of 16° to 26°. Perfused isolated dog (41, 74) and bullfrog (81) kidneys have been cooled to 0° and 6°, respectively, while living alligators and turtles were cooled to 6° (77). Hypothermia has been induced by immersion in ice water, application of ice packs, refrigeration blankets, or low-temperature rooms. Only one worker has utilised extracorporeal blood stream cooling (2), with the animal's heart providing the propulsive power for the circulation.

All this activity does not seem to have yielded much consistent information, however. There is universal agreement (2, 13, 17, 22, 41, 74, 77, 78, 80, 86, 88, 114, 116, 117, 119, 127, 146, 147) that the GFR and RBF fall with cooling, and this out of all proportion to the generally seen concomitant fall in arterial blood pressure, (13, 22, 86, 88, 114, 117, 119, 127). While many workers found the rate of urine production to rise with cooling (4, 74, 80, 88, 146, 147), more have found it to fall (2, 13, 17, 22, 77, 78, 114, 116, 127, 166). Urine concentration, as measured by urinary osmolality (80), urea (78), phosphorus (147), inulin (2) or creatinine (77, 147), concentrations, is said to fall; these studies however have been fragmentary. Some workers have found urinary sodium and chloride concentrations to approach serum levels at low temperatures (74, 77, 81, 146); others

have found no alteration in sodium excretion with cooling (116, 119). Tubular reabsorption of sodium and water has been said to remain unaltered (22), or to fall (41, 74, 78, 80, 88, 117, 119, 146, 147). Proteinuria has been noted in slight amount (17), never, (74), erratically (77), or only on rewarming (147). Several workers have reported glycosuria at low temperatures (77, 78, 88, 147).

Estimations of recovery of renal function on rewarming have yielded similarly discrepant results. These have been based on simple observations of urinary sodium or chloride concentrations, serial clearance studies, or microscopic examination of rewarmed kidneys. Many believe hypothermia to produce no discernible ill effect on the kidneys (4, 13, 17, 115, 127); others find complete recovery of function but only after a variable lag period of up to 24 hours after rewarming (116, 117, 119), and some few have found renal function to be permanently impaired (2, 77).

CHAPTER 11.

PRELIMINARY CONSIDERATIONS.

On reviewing, in 1960, the literature dealing with renal function during hypothermia, two facts stood out prominently. Firstly, renologists had not as yet taken advantage of Gollans principle (67) of combining hypothermia with extracorporeal circulation, so as to attain in the intact animal temperatures as low as those possible in isolated kidney preparations. Secondly, the renal handling of electrolytes, other than Na or Cl, had been largely ignored. In so far as much of the body of knowledge concerning renal mechanisms is built upon observed changes in urinary electrolyte concentrations, under various stressful conditions, it seemed to me that correction of these two omissions might be expected to yield information of value.

Almost all who had previously experimented with the effects of cold upon the kidney, in the intact animal, had used the rectal temperature as their reference point, equating it with the deep body temperature. There was ample evidence that this confidence was justified. A number of workers (21, 78, 114, 146) had compared 'core' temperatures

(measured at various sites) with that of the rectum, or colon, and found little difference between the two. The rectal temperature was usually 0.5° (11¼) to 1°-2° (5) lower than the former. Hong (78) found that while there was a measurable temperature difference between the oesophagus, and colon or kidney, the difference between the latter two was negligible.

This concordance of 'core' and rectal temperatures, as observed with the use of the slow acting surface-cooling techniques, does not hold however when hypothermia is induced rapidly, as with extracorporeal circulation and blood stream cooling. Differences as great as 20° have been found between rectal and heart temperatures (65). Young et al found the rectal to lag as much as 15° behind the oesophageal temperature (185) while Shields and Lewis (153) found this relationship to be quite unpredictable. Stupfel and Severinghaus (164) investigated the problem, and were able to show that cardiac temperatures approximated most closely (almost exactly) with the lower oesophageal temperature. Severinghaus (152) went on to state that "organs with high perfusion, such as brain or kidney, maybe assumed to follow the heart temperature passively".

As a study of renal function precludes the traumatic placing of temperature probes within the substance of the kidney, I was happy to go along with this proposition, and assume the lower oesophageal temperature to be identical with that of the kidneys.

The determination of GFR and RBF, by clearance techniques, demands a constant plasma level of the substance measured (159). The usual procedure is to administer, intravenously, a solution containing, for example, creatinine, utilising a constant infusion pump. With a constant rate of urinary loss, the plasma creatinine level soon reaches a plateau, and reproducible clearance levels can be got. The induction of hypothermia however is complicated by a falling GFR (cf Chapter 1). The rate of renal elimination of creatinine must then diminish. In consequence of this, a constant intravenous infusion will now not maintain, but rather progressively elevate, the plasma creatinine level. Calculated clearance values then become unreliable.

In order to overcome this difficulty, Page (127), while cooling dogs to rectal temperatures of 25°-20°, progressively slowed the rate of infusion as the temperature dropped. Blatteis and Horvath (22), during similar experiments, found that slowing the constant

infusion pump at 30°, to half the original rate, sufficed to keep plasma creatinine levels constant. Bettge et al (17) obtained satisfactory results by reducing the rate of infusion at 25°, to one quarter of that used at 37°. All these experiments concerned dogs, cooled by surface techniques, to rectal temperatures of 25°-20°.

The great majority of published papers, interestingly, make no mention either of this difficulty in technique, nor of their attempts to cope with it, if any.

The use of an extracorporeal circulation introduces a further complication: a litre or so of creatinine - free blood is abruptly introduced into the animals circulation. A sudden drop in plasma creatinine concentration can therefore be expected immediately after institution of cardiac bypass, in dogs previously creatinine-loaded. Just how long a time must elapse for equilibration of body water creatinine, after this dilution, is unknown. Meanwhile the shock of institution of bypass, coupled with the trauma of surgery and continueing anaesthesia, may be lowering the GFR and RBF progressively, while the constant intravenous infusion continues uninteruptedly.

Add the effects of cooling, to those of the

institution of cardiac bypass, and the position 'clearance-wise' becomes untenable. It is just not possible to so regulate the rate of intravenous infusion that constant plasma levels can be knowingly maintained. On the other hand, some estimate of GFR, even if only approximate, seemed essential. I decided therefore to dispense with the use of a constant infusion pump, as being an unnecessary complication in an already complex experimental set up, and elected to use merely the ordinary clinical (Baxter) drip set, where-in the infusion rate is controlled by means of a simple screw clamp. What success greeted this manoeuvre will be evident later.

One practical problem remained; would cooling the dogs to 10° not dry up the flow of urine? There was a good deal of evidence to suggest that this might occur. Several investigators had found that hypothermia lead to complete cessation of urine flow, usually at rectal temperatures of 25°-20° (2, 3, 13, 17, 78, 127, 138). These observations were based on experiments with dogs, rats, ground squirrels and rabbits, all rendered hypothermic by surface or extracorporeal blood stream cooling techniques (in the latter case, without the benefit of assisted circulation). Others, in similar experiments, had noticed simply a great reduction in urine flow rate,

without actual stoppage (22, 77, 114). There were however almost as many testimonies to the contrary (cf Chapter 1). Outstanding among these, were experiments on isolated perfused dog kidneys (18, 74); our use of an extracorporeal circulation would make our experiments approximate most closely to these, and it was reassuring to note the marked rise in urine flow rate in these preparations, as cooling progressed.

In any event, all we could do was to cool the dogs and note what happened. Hong (78) had remarked that any infusion of fluid during hypothermia augumented urine flow; accordingly I decided to run in 10% dextrose water intravenously during the hypothermic period.

CHAPTER III.

DOG A.

AIM.

In this, the first experiment of the series, our aim was simply to ascertain the approximate rate of urine flow in the profoundly hypothermic dog - as so rendered by our technique. The results would dictate the feasability and scope of subsequent investigations.

EXPERIMENTAL PROCEDURE:

The dog was weighed, and anaesthetised. Two intravenous infusions were then commenced, one of 10% Dextrose-water, the other of 3.2 G creatinine in a litre of 5% dextrose water. (The latter was allowed to run in at about ¼ ml. per minute) Immediately thereafter, a priming dose of 0.5 G creatinine was given intravenously.

We then attempted to catheterise the dogs bladder. None of us had had any experience in this manoeuvre, and after about 20 minutes of futile endeavour, we decided to cut through the anterior abdominal wall and then catheterise both ureters via the bladder. This was done, and the catheters were tied firmly into place. The distal ends of the catheters were fed via the urethra, to the exterior, and the bladder and anterior abdominal wall repaired.

The surgeon then carried on with the operative procedure necessary for the institution and maintenance of cardiac bypass and hypothermia (cf Appendix B). By the time all was ready, an hour had elapsed, and the first urine sample collection was begun.

One urine and one blood sample were collected to serve as controls, and then cardiac bypass and cooling were begun. The dog was cooled to an oesophageal temperature of about 11° and the extracorporeal circulation then clamped off. The dog lay, apparently lifeless, with neither circulation nor respiratory movement, for 32 minutes, before the circulation was again instituted, and rewarming begun. When the oesophageal temperature reached 35°, bypass was discontinued and the dogs heart allowed to resume its normal function.

Continuous urine and many blood samples were collected during and for 70 minutes after, the hypothermic period. The arterial blood pressure, and lower oesophageal temperature, were noted frequently. Blood samples were collected in heparinised test-tubes. Immediately the experiment was concluded, serum was separated by centrifugation. Urine sample volumes were measured to the nearest 0.1 ml. The osmolality and creatinine concentrations of all urine and serum samples were then

determined, as well as the protein content of each urine sample.

Creatinine clearances were calculated in the usual way, as: $C_{cr} = \frac{U\ V}{P}$ where U = urinary concentration of creatinine

V = urine volume per minute

P = plasma concentration of creatinine.

No allowance was made for 'delay time'. The plasma creatinine concentrations used in these calculations were those estimated by simple proportion to have pertained at the midpoint of each urine collection period.

The percentage excretion of filtered water, was calculated as:

$$\% \text{ excretion } H_2O = \frac{V \times 100}{C_{cr}}$$

where V = urine volume per minute

C_{cr} = creatinine clearance per minute.

RESULTS:

The results of the experiment are given graphically in the accompanying figure. Details of both experimental procedure and results can be found at the end of this chapter.

The urinary flow rate fell immediately bypass and cooling were instituted, only to increase slightly at 15^{0}-10^{0}. During the first minutes of the ensueing period,

when the extracorporeal circulation had been stopped, a few drops (1.5 ml) of urine continued to drip out of the catheters. On resumption of bypass, urine promptly appeared again, and as the temperature rose, so did the urinary flow rate, to soon exceed the control level. Restitution of the dogs normal circulation was marked by a still greater rate of urine flow to some four times the original.

Urinary osmolality fell steeply from the control level of 1032 m Osm./litre, to 524 m Osm/L. at 15°-10°; rewarming did not reverse this, the urinary osmolality remaining at 480 mOsm./L. to 429 mOsm/L thereafter.

The arterial blood pressure dropped sharply with commencement of bypass and cooling, to reach 59 mm Hg. Only 3 readings were recorded during rewarming; these indicate that the blood pressure remained low (60 mm Hg) even on regaining 30° to 34°. No blood pressure readings were taken after restitution of the normal circulation.

The creatinine clearance fell markedly during bypass and cooling, with a slight rise again at 15°-10°. Rewarming reversed this fall, but tardily, so that the creatinine clearance only approached that of the control level about sixteen minutes after the normal circulation

had been resumed.

The percentage excretion of filtered water rose from an initial 2.3%, to 15.9%. at 11°; on rewarming, it fell again but only to 10.3%.

Proteinuria was present throughout. Urine samples 5 and 6 were not tested. Urinary protein concentration fell during the induction of hypothermia from 20 mg./100 ml to 10 mg./100 ml. On rewarming there was a transitory rise to 25 mg./100 ml, after which it settled to 15 mg./100 ml.

Inspection of the sera obtained from blood samples 1 to 9, showed progressive reddening. The haemoglobin content of the serum was not measured.

INTERPRETATION:

It will be recalled that by the time the first, control, urine collection was made, two intravenous infusions - one of 10% dextrose water - had been running for an hour, at a combined rate of about 8 ml/minute. Despite this, the urinary flow rate during period 1 was but 0.6 ml. per minute, and the urine of relatively high osmolality (1032 mOsm./litre). The creatinine clearance was 25.9 ml. per minute, or 1.7 ml/kg. body weight/minute; reference to Appendix C reveals this to be considerably

below accepted mean normal values (but just at the lower limit of the normal range found by Asheim et al. in their series of 32 dogs). These data suggest the presence of glomerulo-tubular imbalance i.e. normal tubular function but a lowered glomerular filtration rate. As the dog had by this time suffered major abdominal and thoracic surgery, without the benefit of replacement of blood loss, this explanation might be accepted as the most likely.

The fact that the creatinine clearances from period 8 onwards are practically at the control level, implies that the intervening periods of hypothermia with cardiopulmonary bypass did not lead to sustained further diminution in renal function. The diuresis and persistently low (relatively) urinary osmolality at this time, are then presumably an expression of the continued intravenous infusions. It is of course equally possible to argue that the latter phenomena are due to diminished tubular function in the presence of an unaltered GFR. The calculations of the percentage excretion of filtered water, pre - and post - hypothermia, do not help one to choose between these alternatives.

The phenomena noted during the periods of cardio-pulmonary bypass and hypothermia, are naturally the

resultant of both these factors; their effects cannot be evaluated separately.

COMMENT ON TECHNIQUE:

Scrutiny of our detailed results revealed several errors in technique.

It is difficult to understand why none of us could catheterise the (anaesthetised) dog's bladder. As it happened, it was just as well that we did catheterise both ureters directly, in view of the small quantities of urine obtained at low temperatures. The usual method of urine collection, with bladder washouts of distilled water, or saline, and air, would have been a source of great error in evaluating our results.

The serum creatinine concentrations behaved, on the whole, as predicted (cf Chapter 11) rising during the bypass-hypothermic period, when the GFR fell. Later, when the GFR was restored to the control level, the serum creatinine concentration became relatively stable. The rise between blood samples 1 and 2 was much less than that between bloods 2 and 3; this presumably was due at least partly to the introduction of the uncreatinised blood from the extracorporeal circuit. The rise in serum creatinine concentration in blood sample 3, can only mean that the rate of creatinine infusion was too fast. It will be noted that blood no. 4 has approximately the same creatinine concentration as blood

No. 3, while blood No. 5 shows a marked rise. On reflection, the cause of this anomaly was painfully obvious; it should have been anticipated and so avoided. In our ignorance of what to expect, our experimental design had been largely a mimic of the surgeons' everyday clinical operative procedure; hence the period of prolonged cessation of circulation during profound hypothermia, which period would normally be used for definitive cardiac surgery. In so far as there was no circulation when blood No. 4 was taken, its creatinine content had of necessity to approximate to that of sample No. 3; on the other hand, the intravenous creatinine infusion continued throughout period No. 5, so that immediately the circulation was resumed the pool of creatinine rich blood at the infusion site was swept into the circulation, to result in the much higher creatinine concentration of blood No. 5. Clearly, this interim period of circulatory stagnation had to be abolished in subsequent experiments, if we were to hope for reasonably stable serum creatinine concentrations. Alternatively, one could stop the creatinine infusion during this period.

Blood pressure readings were recorded only after the introduction of cardiopulmonary bypass and hypothermia; this makes it difficult to evaluate

the concomitant drop in GFR. These readings needed to be recorded before and after this period.

Evaluation of urinary osmolality, as evidence of renal concentrating ability, is not complete without a knowledge of serum osmolality. A 10% dextrose water solution, as infused here, has an osmolality of 550 mOsm/litre, twice that of normal serum. In an experiment such as this, where hyperosmotic 10% dextrose water is being continually infused, while urine formation is diminishing, the serum osmolality maybe expected to rise. Indeed, the serum osmolality of 327 mOsm/L as seen in the control period 1, before the induction of bypass and hypothermia, maybe a manifestation of this phenomenon. It is impossible then to state whether the final urinary osmolality of circa 450 mOsm/L., in periods 8 to 12, was hyper, - iso -, or even hypo - osmotic to the serum at that time. In future experiments serum osmolality would need to be recorded throughout.

CONCLUSION:

The aim of the experiment was to determine whether or not sufficient urine would be forthcoming during profound hypothermia, to enable multiple chemical determinations to be made on these samples. The observed urinary flow rates of 0.26 and 0.27 ml./minute (during the

'cold' periods 4 and 6) were, I thought, just adequate. We could now proceed to more definitive studies.

Several practical points had come to light, all of which had a bearing on projected future experiments. Rather than bladder catheterisation, urine should be collected directly from the ureters; the rate of intravenous creatinine infusion should be less than that adopted here; arterial blood pressure readings needed to be recorded before and after, as well as during the introduction of cardiopulmonary bypass and hypothermia; and serum osmolality needed to be measured throughout the experiment. Finally, and most important, distinction had to be made between the renal effects of bypass and those of hypothermia.

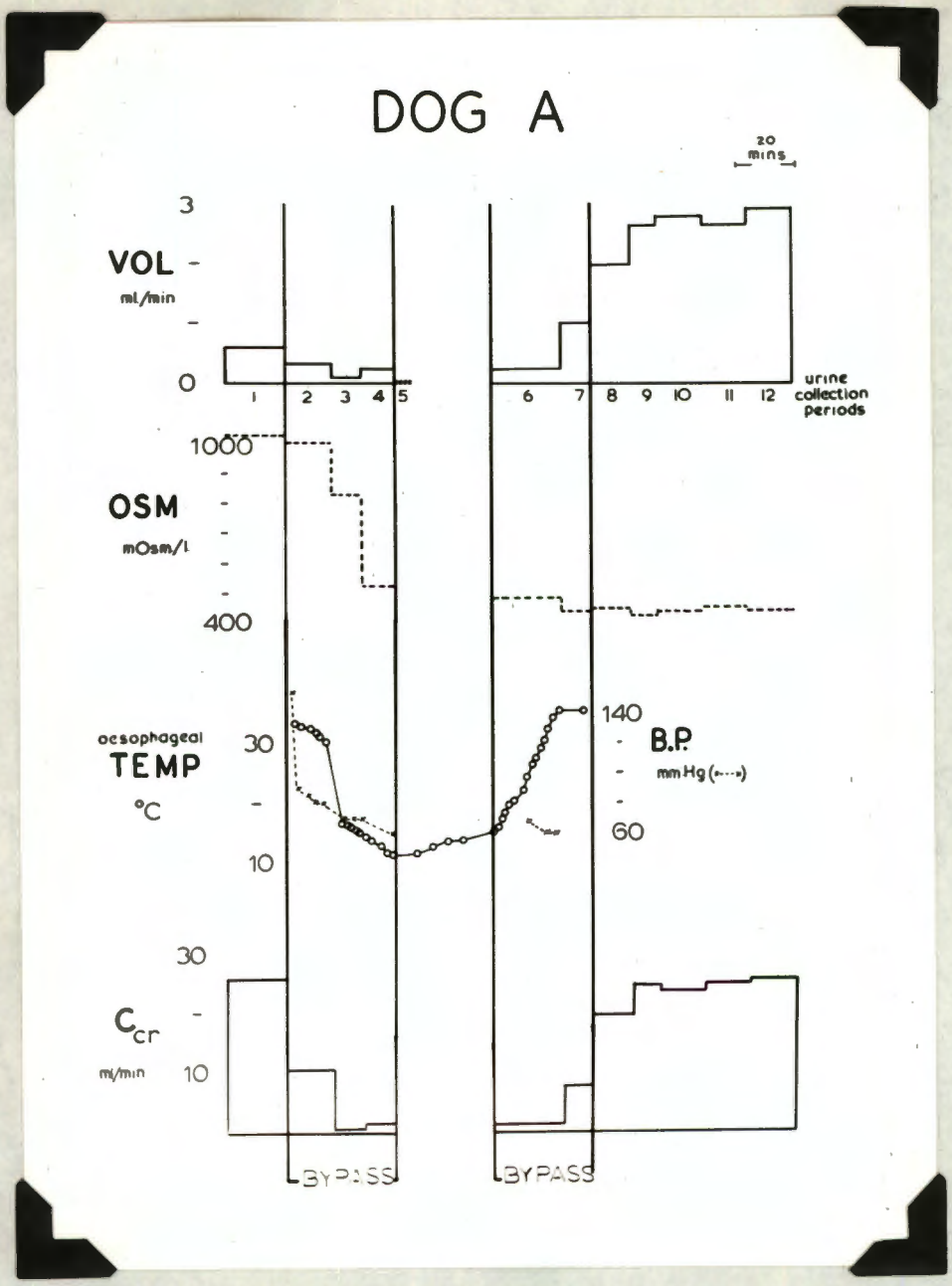

DOG A
20 mins
3
VOL
ml/min
0
1 2 3 4 5 6 7 8 9 10 11 12
urine collection periods
1000
OSM
mOsm/l
400
oesophageal
TEMP
°C
30
10
140
B.P
mm Hg (•---•)
60
30
C_{cr}
ml/min
10
BYPASS
BYPASS

FIG. 1.

- EXPERIMENTAL DATA ON DOG A. -

Weight: 15.0 Kg

Infusions: (1) 10% dextrose water.
(2) 3.2 G creatinine dissolved in 1 litre of 5% dextrose-saline, run in at about 4 ml./minute. Begun at 12.29 p.m.

Priming Dose of creatinine: 0.5 G, given at 12.30 p.m.

Equilibration period: 1 hour.

Urine collection via polyethylene catheters inserted into ureteral orifices via bladder; (bladder opened through transperitoneal, anterior abdominal, midline incision).

Times of blood and urine collections:

In this and subsequent experiments, the circled figures refer to sample numbers; the other figures refer to times of collection. The figures pertaining to blood samples are given above the continuous series of squares; the latter represent consecutive urine collections.

Blood Samples:	① 1.38 pm	② 1.58 pm	③ 2.18 pm	④ 2.38 pm	⑤ 2.58 pm	⑥ 3.22 pm	⑦ 3.45 pm	⑧ 4.14 pm	⑨ 4.34 pm		
Urine collection periods:	1.27 p.m. to 1.47 ①	to 2.02 ②	to 2.12 ③	to 2.23 ④	to 2.55 ⑤	to 3.18 ⑥	to 3.28 ⑦	to 3.41 ⑧	to 3.50 ⑨	to 4.05 ⑩	to 4.20 ⑪	to 4.35 ⑫

Time (p.m.)	Oesophageal Temperature °C	Arterial Blood pressure mm Hg.	REMARKS.
1.50	33.4	155	
52	33.0	90	
55	32.7	85	
57	31.9		Perfusion: 800 ml/minute
58	31.0	80	
2.00	30.1	80	
.05	16.7		
.07	16.3	70	
.08	16.0		Perfusion: 1000 ml/minute
.10	15.4	70	
.11	15.0		
.13	14.4	70	Perfusion: 800 ml/minute
.15	13.9		
.16	13.6		
.18	12.9		
.20	11.8		
.22	11.5		
.23	11.0	59	
.30	11.8		
.35	12.9		
.40	13.4		
.45	13.8		
.55	14.8		

Time (p.m.)	Oesophageal Temperature °C	Arterial Blood Pressure mm Hg.	REMARKS.
57	16.0		
58	17.2		
59	18.5		
3.00	19.8		
.02	20.2		
.05	22.1		
.06	24.4	68	
.08	26.3		
.09	27.5		
.11	29.1		
.12	30.6	60	
.13	32.4		
.15	34.1	60	
.17	35.2		
.25	35.4		
			Last stitches at 4.48, when all wounds closed.

OBSERVED DATA:

URINE

Urine No.	1	2	3	4	5	6	7	8	9	10	11	12
Volume (ml.)	12.0	5.0	1.0	3.0	1.5*	6.0	10.0	26.0	24.0	42.0	40.0	44.0
(mOsm/L) Osmolality	1032	1002	8.28	524	-	484	440	447	429	442	458	440
Creatinine mg./100 ml.	431.9	349.1	75.0	95.7	-	135.3	175.8	208.6	186.9	169.0	181.9	176.7
Protein * mg./100 ml.	20	15	15	10	-	-	25	15	15	15	15	15

* Urine flow stopped completely in period 5. The 1.5 ml dripped slowly out of the catheters for the first few minutes only.

* to nearest 5 mg/100 ml.

BLOOD.

Sample No.	1	2	3	4	5	6	7	8	9
Osmolality (mOsm/L)	327	-	-	-	-	-	-	-	-
Creatinine (mg/100 ml.)	10.0	11.55	15.26	14.31	22.24	22.50	20.34	19.48	20.52

DERIVED DATA:

Urine Collection Period	1	2	3	4	5	6	7	8	9	10	11	12
Urine Vol. (ml/minute)	0.60	0.33	0.10	0.27	0.05	0.26	1.00	2.00	2.67	2.80	2.67	2.93
Midperiod serum creatinine (mg/100 ml.)	10.0	11.1	13.2	15.2	14.7	22.3	22.4	21.4	20.3	20.0	19.5	20.2
Creatinine clearance (ml/min.)	25.9	10.3	0.6	1.7	-	1.6	7.8	19.5	24.6	23.7	24.9	25.6
% excretion of filtered H_2O	2.3	3.2	16.7	15.9	-	16.3	12.8	10.3	10.9	11.8	10.7	11.4

CHAPTER IV.

DOG B.

Having ascertained that urine was produced by the cold kidney, the next problem was to decide what to measure in this urine, so as to obtain as much information on renal function during hypothermia as possible. Our experience with Dog A had given us reason to expect urine flow rates of 0.2 to 0.3 ml. per minute, at oesophageal temperatures of 10° to 15°. Urine collection periods of 10 to 20 minutes duration would therefore yield aliquots of 2 to 6 ml. These figures set an upper limit to the number of chemical investigations feasible.

Every measurement, even the elementary one of urine volume, I would have to do myself. This set another limit, that of time, on the complexity of any projected study. Yet it was obviously desirable to follow as many parameters of renal function as possible, andthese preferably to encompass, functionally, the whole length of the nephron. That is to say, I thought it of more value, and interest, to note, for example, changes in phosphate reabsorption and K secretion - thus yielding information concerning both proximal and distal tubules - than to study an equal number of only distal tubular functions.

My final decision was to follow changes in urinary volume and creatinine clearance - and so derive changes in glomerular filtration rate; Tm PAH and inorganic phosphate reabsorption - so as to measure both secretion and reabsorption in the proximal tubule; and Na, Ca, Mg and K concentrations, and osmolality - and so obtain information on 5 different aspects of distal tubular function. All the chemical estimations necessary had the essential merits of being both technically simple, and rapid to perform. (cf Appendix D.)

The plan of the experiment would be as for Dog A, but amended as suggested by our experience, that is, renal function would be studied during normothermic cardio-pulmonary bypass, <u>before</u> cooling, so as to differentiate the effects of these two procedures on the kidney. We decided to dispense with the period of prolonged circulatory and respiratory arrest during profound hypothermia, as this not only increased the difficulty of attaining constant serum creatinine levels (cf Dog A) but also because such a procedure actually set additional problems, viz: the renal effects of (1) <u>prolonged</u> hypothermia, and (2) circulatory arrest during profound hypothermia. A period of normothermic bypass was to succeed the hypothermic period, to provide evidence of

reversibility of hypothermic effect (if any). Finally as the surgeons were concerned not only with the renal effects of hypothermia, but also with those of cardio-pulmonary bypass, we were to extend our experimental observations prior to and beyond the total period of bypass.

EXPERIMENTAL PROCEDURE:

With the following exceptions, the procedure followed was identical to that for Dog A.

No time was wasted attempting to catheterise the bladder. The ureters were catheterised, directly, via an anterior abdominal incision, and the catheters tied firmly into place.

The creatinine content of the sustaining intravenous infusion was lowered, from the previous 3.2G, to 1.5G per litre. A large dose (2.4G) of para-amino-hippurate was added to this sustaining infusion. Immediately this was begun, a priming dose of 0.5G creatinine and 1.8G para-amino-hippurate was given intravenously. Equilibration was assumed to be complete 32 minutes later, when the first urine collection was begun.

The ureteral catheters were clamped off for a few minutes between urine collection periods 2 and 3, 6 and 7

and 15 and 16. This was an impromptu attempt to perform stop-flow studies before, during and after hypothermia.

Urine and blood collections continued until 52 minutes after resumption of normal circulation.

All urine specimens collected were measured for volume, osmolality, creatinine, para-amino-hippurate, sodium, potassium, calcium, magnesium and phosphorus concentrations. The degree of proteinuria was also determined. Similar measurements, (other than for volume and protein content) were made on the serum samples. The hemoglobin content of the last serum sample taken (No. 11) was also determined.

The percentage excretion of the various electrolytes (Na, K, Ca, Mg, P) was calculated as equal to:

$$\frac{(Ux.V}{Cer.Sx)} \times 100$$

Where Ux = urinary concentration of the electrolyte in question.
V = urine volume, in ml./minute
Cer = creatinine clearance, in ml./minute
Sx = serum concentration of the electrolyte in question, at midpoint in time of the urine collection period. Where necessary, this concentration was calculated by simple proportion from actual preceding and succeeding serum electrolyte concentrations.

The percentage excretion of filtered water was calculated as:

$$\frac{V}{Cer} \times 100$$

No attempt was made to determine the ultrafilterable fractions of Ca or Mg; the total serum concentrations of these electrolytes were used in the calculations of percentage excretion. This willbe discussed later (cf Chapter 16).

A graphic plot was made of oesophageal temperatures against time. The average oesophageal temperature, for the duration of each urine collection period, was then derived by simple proportion.

The clearance of PAH, or C_{PAH}, was calculated in the usual way:

$$C_{PAH} = \frac{PAH_u \cdot V}{PAH_s}$$

Where PAH_u = urinary PAH concentration

PAH_s = serum PAH concentration

V = urine volume in ml./minute.

The Tm PAH was calculated as equal to:

$$V \times PAH_u - (GFR \times PAH_s \times 0.92)$$

where V = urine volume, in ml./minute

PAH_u = urinary concentration of PAH in mg./ml.

GFR = glomerular filtration rate, here equated with the creatinine clearance, in ml/minute.

PAHs = serum concentration of PAH, as found or calculated for the midpoint in time of the urine collection period.

0.92 = The filterable fraction of serum PAH (168).

RESULTS:

The results are shown graphically in the accompanying three figures (Fig. 2, 3, 4). Details of experimental and derived data are given at the end of this Chapter.

Urinary flow rate and osmolality: blood pressure, Cer and Tm PAH. (Fig. 2)

With the commencement of normothermic cardiopulmonary bypass (Period 2) the urine flow rate dropped, but not as markedly as did the Cer and TmPAH. Urinary osmolality rose slightly, from 469 mOsm./L to 480 mOsm/L. The arterial blood pressure remained unaltered. Period 3, the last 6 minutes of the half hour of initial normothermic bypass, showed a further drop in urine flow rate, Cer and TmPAH. The urinary osmolality had by now risen appreciably, from 480 to 601 mOsm/L. The B.P. meanwhile stayed steady at 110 mm Hg.

Blood stream cooling was initiated at the commencement of urine collection period 4. There was an immediate change in all parameters measured. Urine flow rate rose abruptly from 1.0 to 4.3 ml./minute. This was accompanied by an equally dramatic rise in Cer, which rose from 34.7,

to exactly the prebypass control level, of 104.2 ml./minute. Tm PAH rose too, but not as markedly. Urinary osmolality fell to 460 mOsm/litre. The B.P. remained unaltered.

As the oesophageal temperature fell further (periods 5, 6) so did the urinary flow rate, osmolality, Ccr and Tm PAH. The urinary flow rate did not however fall as low as during period 3. By period 7, urine osmolality had fallen to 321 mOsm/litre, only slightly above the serum osmolality of between 303 mOsm./L. and 317 mOsm/L. The Ccr and Tm PAH fell very steeply, the latter achieving negative values. With cooling, the C_{PAH} fell from levels initially higher than those of the Ccr, to almost identical values at 11.9° (period 6). The B.P. fell pari passu with the oesophageal temperature.

Rewarming, periods 7 to 11, led to little change in urine flow rate, except for a transitory drop in period 10. Urinary osmolality rose from 321 mOsm./L to 445 mOsm./L then fell finally to 401 mOsm/L. The Ccr rose slowly and irregularly to 68 ml./min. in period 11. The Tm PAH at first gave negative figures; by period 11 it was still less than during period 3 (2.5 mg/min. as compared to 4.4 mg./min.) The B.P. remained low, but rose from 55 to 70 mm Hg just before bypass was discontinued.

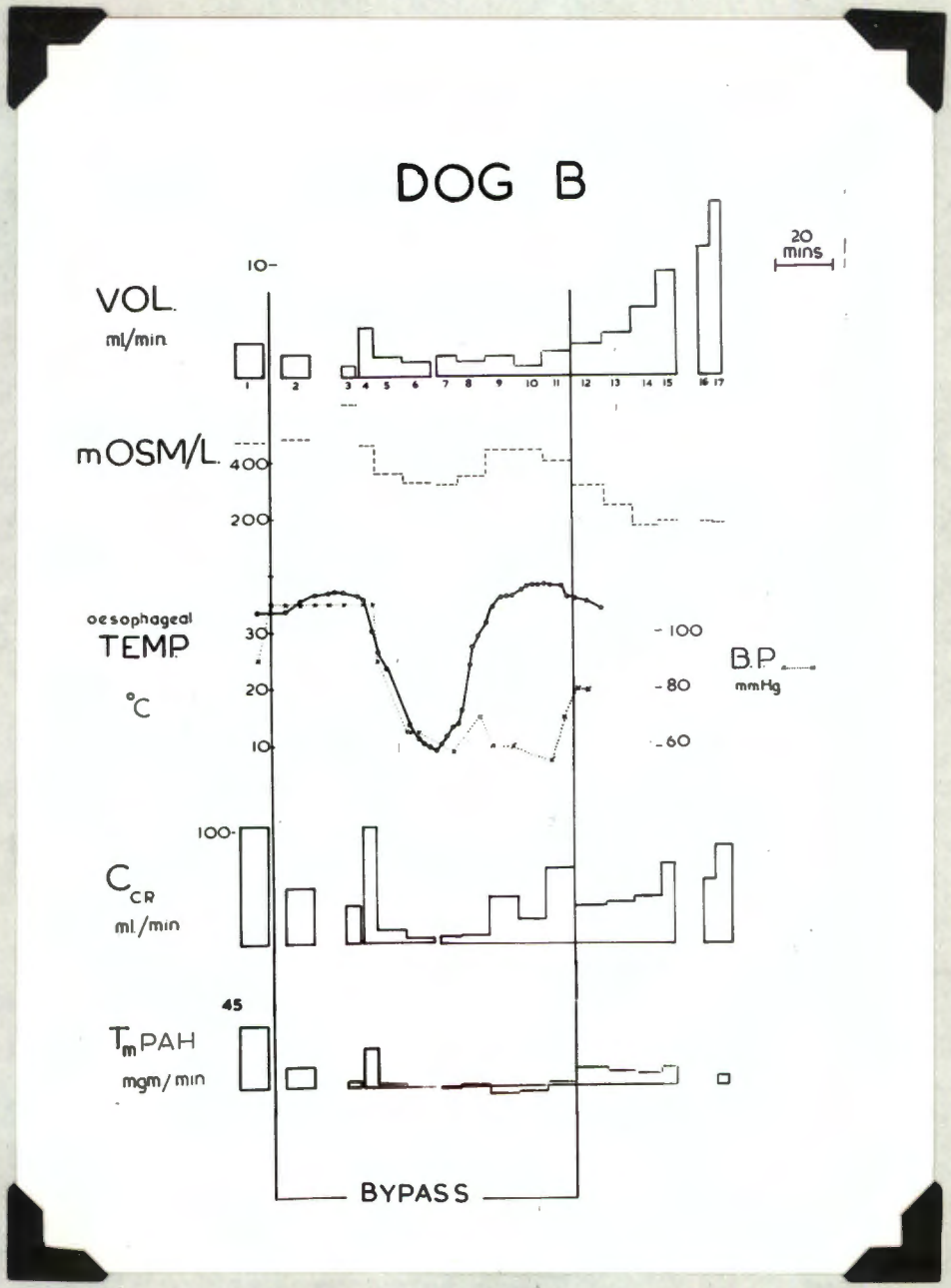

DOG B
20 mins
VOL.
ml/min
10-
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
mOSM/L.
400
200
oesophageal
TEMP
°C
30
20
10
100
80
60
B.P.
mmHg
C CR
ml/min
100-
T mPAH
mgm/min
45
BYPASS

Fig. 2.

Normal circulation was resumed from periods 12 onwards. Urine flow rate increased progressively, so that by period 17 it was 15.3 ml./minute, five times greater than the initial prebypass rate. Urinary osmolality fell, concomitantly, to reach 182 mOsm/L. during period 17. The Ccr fell abruptly, after cessation of bypass, but soon rose thereafter; it did not however regain its pre-bypass value by the time the experiment was concluded. Tm PAH remained low. The B.P. remained at 80 mm Hg after restitution of normal circulation.

Urinary Electrolyte and PAH concentrations. (Fig. 3)

Marked changes occurred in urinary electrolyte and PAH concentrations during the course of the experiment.

Introduction of normothermic bypass led to a steep and progressive drop in urinary Na concentration, from 117.5 meq./L. to 4.3 meq./L. Subsequent cooling reversed this trend, so that by period 6 (mean oesophageal temperature of 11.9°) the urinary Na concentration approximated to that of the serum. Rewarming promptly returned this figure to 5 meq./L.; subsequent urine samples, both before and after restitution of the normal circulation, showed a slow irregular increase to about 50 meq./L.

Little change in urine K concentration was brought about by normothermic bypass, but hypothermia rapidly reduced it from 16.3 meq./L. to 3.0 meq./L., just above the serum level of 2.24 meq./L. (period 6). On first rewarming (period 7), while the mean oesophageal temperature rose from 11.9° to 12.1°, the urinary K concentration fell further, to 2.7 meq./L. ; thereafter it rose rapidly to 15.3 meq./L. (period 11). After bypass was discontinued, urinary K concentration fell again, progressively, to 2.2 meq./L. (period 17).

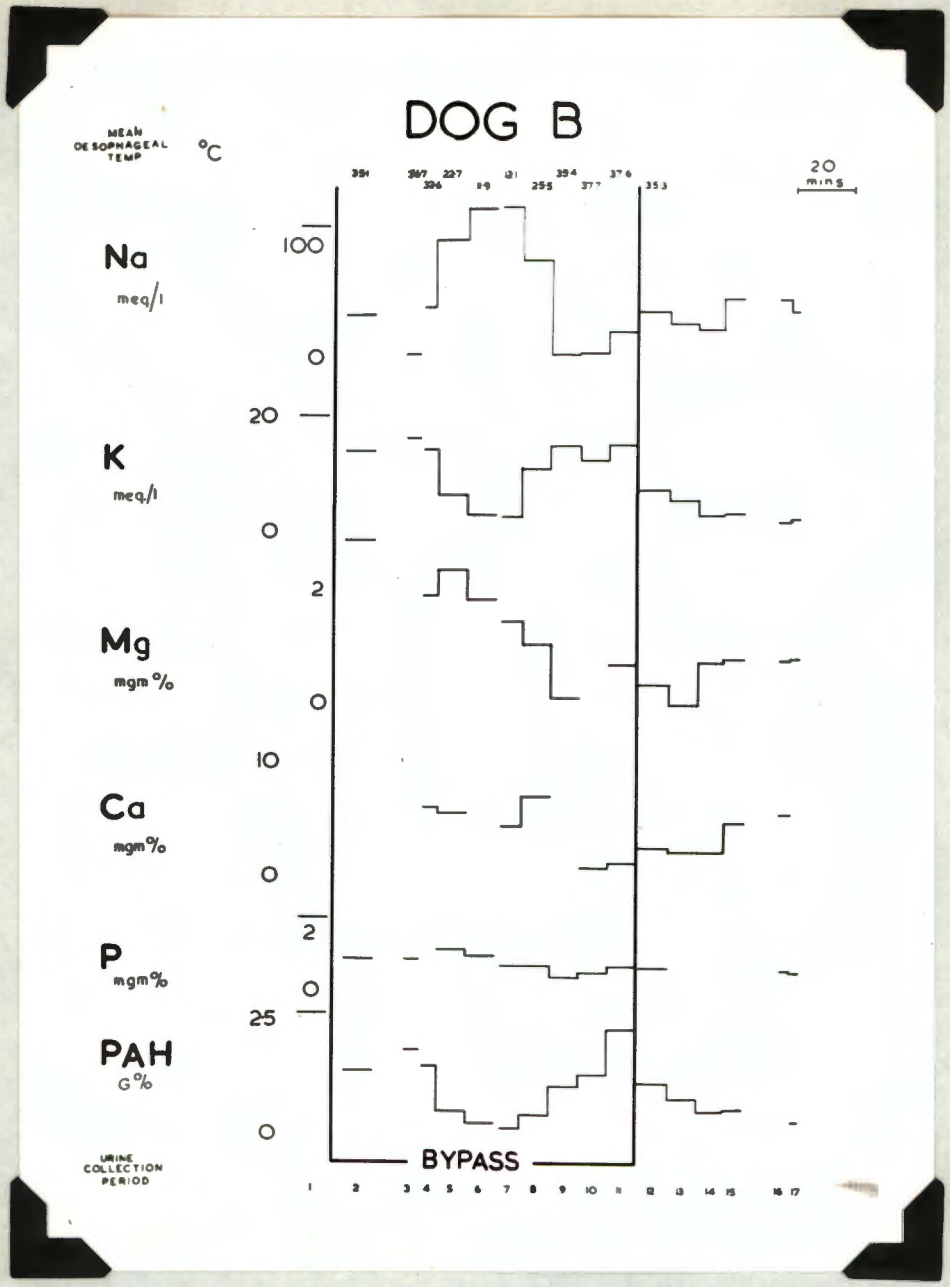

DOG B
MEAN OESOPHAGEAL TEMP °C
20 mins
Na
meq/l
100
0
K
meq/l
20
0
Mg
mgm%
2
0
Ca
mgm%
10
0
P
mgm%
2
0
PAH
G%
25
0
BYPASS
URINE COLLECTION PERIOD
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

Fig. 3.

The urinary PAH concentration followed a course very similar to that of the urinary K concentration, reaching a low of 140 mg/100 ml. during period 7 (serum PAH concentration 60.7 mg/100 ml.). As with K, the urinary PAH concentration was lower during period 7 than period 6, despite the fractional rise in mean oesophageal temperature.

For technical reasons (cf discussion) I was unable to determine Mg and Ca concentrations on all urine samples. The data given for these electrolytes are therefore fragmentary, and it is not possible to follow their concentration changes throughout the alternating periods of normo- and hypo-thermic bypass. Some figures are worthy of note however. During profound hypothermia, the urinary Mg concentration was 1.84 mg/100 ml. (period 6) and 1.46 mg/100 ml (period 7), when the midperiod serum Mg concentrations were 1.37 mg/100 ml and 1.26 mg/100 ml respectively. On rewarming, with bypass, urinary Mg concentration fell sharply, to 0.15 mg./100 ml. (period 9) Thereafter, like Na, the urinary Mg concentration rose irregularly to reach a concentration of 0.82 mg/100 ml. during period 17. Urine calcium concentration at 12.1° was 4.47 mg/100 ml. (serum level 6.54 mg./100 ml.), and on rewarming, with

bypass, this fell to 0.78 mg./100 ml. and 1.33 mg/100 ml (with serum levels 7.16 and 7.37 mg/100 ml respectively) during periods 10 and 11. On resumption of normal circulation, this rose slowly to 5.45 mg/100 ml (period 16).

The degree of proteinuria: changed significantly during the course of the experiment, and in a manner quite unlike that of the other urinary constituents. The trace of proteinuria detected before and during the initial normothermic bypass, and during period 4 (mean oesophageal temperature 32.6°), disappeared during periods 5, 6 and 7 (mean oesophageal temperatures of 22.7°, 11.9° and 12.1° respectively) only to reappear in period 8 (25.5°). It then rapidly rose to 10 mg/100 ml. (35.4° - period 9), 15 mg/100 ml. (37.7° - period 10), and finally to 35 mg/100 ml. (period 11 - at 37.6°) before dropping abruptly on discontinuation of bypass, to its original level of less than 5 mg./100 ml. (normothermic periods 12 to 16). It then disappeared altogether in period 17.

Percentage Excretion of Filtered Water and Electrolytes. (Fig. 4)

Half an hour of normothermic bypass lowered the percentage excretion of filtered Na from 2.4% to 0.1% Subsequent cooling, produced a rise to 25.9%, at 11.9°. On rewarming, this figure fell again to low levels, reaching 0.2% at 37.7° (period 10). After discontinuation of bypass,

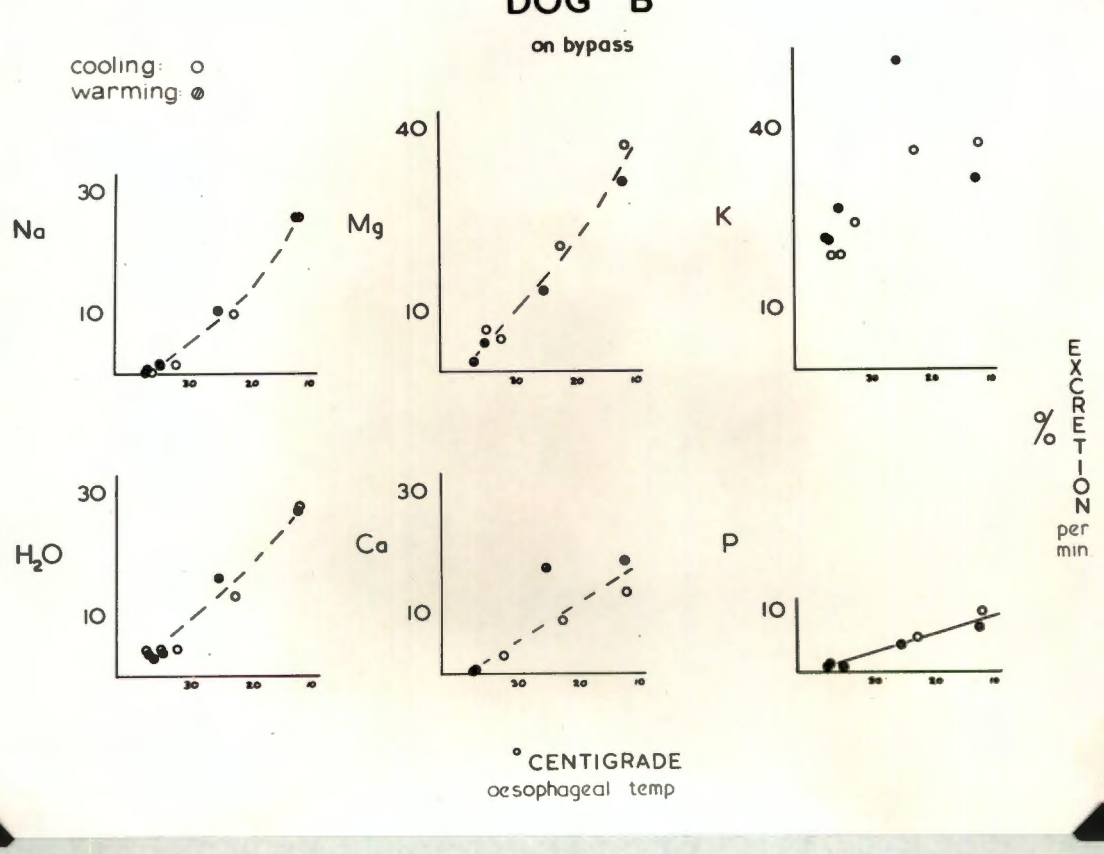

DOG B
on bypass
cooling: o
warming: ø
Na
Mg
K
H2O
Ca
P
40
30
10
% EXCRETION per min
° CENTIGRADE
oesophageal temp

Fig. 4.

the percentage excretion of Na rose to reach 7.7%, and 5.5% during periods 16 and 17 respectively.

The changes in percentage excretion of filtered water brought about by cooling and subsequent rewarming, closely paralled those of Na excretion. During normothermic bypass however water excretion differed from that of Na in that it remained unaltered during this period. After resumption of normal circulation, the percentage excretion of filtered water rose gradually to 20.5% and 17.7%, during periods 16 and 17 respectively.

Variations in percentage excretion of magnesium where measured, were similar to those of Na rising with cooling to 37.2% at 11.9°, and falling to 1.6% on rewarming to 37.6°. After discontinuation of bypass, the percentage excretion of Mg reached 11.8% and 10.7% (periods 16 and 17).

Calcium, like Mg, followed a course similar to that of Na. They differed however in that their percentage excretions at the lowest mean oesophageal temperature attained - 11.9° - were more (Mg), and less (Ca) than that of Na.

The percentage excretion of K, like that of water, did not seem to be significantly affected by normothermic bypass, and rose reversibly with cooling. Unlike the

case with other electrolytes presented so far, a graphic plot of percentage excretion of filtered K against temperature, yielded points too scattered to permit of the construction of a smooth curve.

Urinary inorganic phosphate revealed the same regular reversible increase in percentage excretion with falling temperature, as did the other electrolytes (with the exception of K - cf above). The changes in percentage excretion between 11.9° and 37° were however strikingly less (9.8% to 1.0%) than was the case for the other electrolytes.

Analysis of serial serum samples revealed the following: Osmolality steadily progressed, from an initial 298 mOsm/L., to 315 mOsm/L. Serum creatinine concentration dropped from 3.09 mg./100 ml. to 2.73 mg/100 ml on commencement of bypass, but thereafter rose gradually to reach 5.00 mg./100 ml. during period 7, and stayed between 4.91 mg./100 ml and 5.91 mg/100 ml. thereafter. Serum PAH concentration also dropped on initiation of bypass , from 46.8 mg./100 ml to 34.2 mg./100 ml, and again like creatinine, thereafter rose progressively, to reach 72.8 mg./100 ml. during period 12, before falling to 60 mg./100ml. during period 17. The serum Na concentration showed little alteration, varying irregularly between 137.5 meq./l. and 145.0 meq./l. Serum K dropped from an initial 2.85 meq./l. to 2.20 meq./l at 11.9°, and varied between 2.24 meq./l and 2.43 meq/l thereafter. Serum Ca concentration dropped from 8.82 mg/100 ml to 6.36 mg/100 ml during hypothermia, to rise again to 7.66 mg/100 ml on rewarming. Serum Mg concentration dropped from 1.62 mg/100 ml to 1.24 mg/100 ml with cooling, and rose to 1.35 mg/100 ml on rewarming. Serum inorganic phosphate concentration fluctuated between 3.10 mg/100 ml and 3.8 mg/100 ml, before finally falling to 2.3 mg/100 ml (period 12).

The plasma haemoglobin at the conclusion of the experiment was 54 mg/10 0 ml.

COMMENT:

As had been the case in the first experiment, analysis of our results with Dog B suggested several further refinements of technique.

Urine flow rates were considerably higher than expected. At 11.9°, Dog B passed 1.4 ml. urine/minute, five times more than had Dog A. This relatively abundant urinary flow suggested that in future we need cannulate one ureter only; this could be exposed through a small flank incision and the ureter dissected out retroperitoneally. Not only would this be a less surgically shocking procedure than that adopted hitherto, but also the 'dead space' between kidney and urine collection bottle would be reduced. This, in turn, would make the urinary findings more representative of renal function at the particular temperature of collection.

The serum concentrations of both Creatinine and PAH dropped immediately bypass was commenced, only to rise shortly thereafter. This, of course, was the behaviour that had been anticpated (cf Chapter 11). The rise was much less than had been the case in Dog A; thus confirmed that we had been on the right track in reducing the creatinine concentration of the sustaining infusion. Possibly stopping the infusion altogether at

some point during hypothermia might have lead to the attainment of even more constant serum creatinine and PAH concentrations than obtained here.

The only way to overcome the fall in serum creatinine concentration on initiation of bypass, would be to introduce an arbitrary amount of creatinine into the blood used to prime the extracorporeal circulation. This would then demand further lowering of the sustaining-infusion creatinine concentration, or stopping the infusion at some undetermined point during the initial normothermic bypass period. (The progressive fall in creatinine clearance seen here during normothermic bypass would per se demand a lessening of infusion rate, to keep serial serum creatinine levels constant). In any event, it seemed clear that the guesswork and labour entailed in attempting any such manoeuvre far outweighed any advantages that might accrue therefrom, particularly as the variations in serum-creatinine concentrations observed in Dog B, were, I thought, acceptable for our purposes.

Large variations in percentage excretion of filtered electrolytes and water occurred with changing temprature. However, striking changes were also produced simply by the institution of bypass (compare the findings during

normothermic bypass with those seen during the prebypass period). It follows that the evaluation of temperature effect on the renal reabsorption of water and electrolytes, can be based solely on those findings made during the bypass period itself. Consequently only these data are plotted in Fig. 4.

Calcium and magnesium estimations were not made on several urine samples. The reason for this was simply that no urine was available for this purpose, all having been used in the duplicate determination of the other electrolytes, creatinine and PAH. Scrutiny of the data presented in Fig. 3 convinced me that this routine precautionary duplication of chemical estimations deprived us of more information that it was worth. While one can infer from the data shown, that urinary Ca and Mg concentrations fall on rewarming during bypass, we have no knowledge of their variations before hypothermia was introduced. Renal handling of these electrolytes might have been grossly affected by cooling. The smooth reversible patterns of Na, K and PAH concentration changes suggest that urinary Ca and Mg concentrations might have shown similar variations.

In short, as we were looking for general trends in the renal handling of water and electrolytes (during

profound hypothermia), rather than attempting to establish exact quantitative data, I felt that in future, where urine samples were of limited volume, I would do all chemical estimations just once, and duplicates only on such material as remained surplus.

Inspection of Fig. 3 reveals that at a mean oesophageal temperature of 12.1°, urine Na concentration was higher, and K and PAH concentrations were lower, than those of the preceding period at 11.9°. That is, the trend in concentration changes with cooling seemed to be prolonged into the initial rewarming period, before subsequently undergoing rapid reversal. This might reflect a real delay in renal response to change of temperature; or more likely, be simply a manifestation of a temperature gradient between oesophagus and kidneys. Kidney temperature changes might be lagging a few minutes behind those of the oesophagus.

In either case, if one wished to correlate urinary electrolyte concentrations with temperature, it would then be necessary to keep the temperature constant at various levels for several minutes, rather than to plunge it downwards, and then equally abruptly upwards, as done here.

Fig. 4 shows that at any given mean oesophageal temperature on bypass, the rates of excretion of water and

electrolytes (other than K) during cooling appear to be equal to those that obtain during rewarming. This, if true, implies immediate and complete reversibility of the cold-induced paresis of renal reabsorptive mechanisms. This would be an important conclusion to draw from our experiments. There would be the need then for more points on the graph between the temperature extremes of 37° and 10°. The points shown at ± 25° can be only approximations, as these were derived from urine collection periods 5 and 8, which encompassed temperature ranges of from 28° to 15°, and of 14° to 31.9°, respectively.

These considerations led me to the conclusion that, in future experiments, we should drop the temperature 'step-wise', keeping it constant at various levels for several minutes.

CONCLUSIONS:

The experiment on Dog B had been, on the whole, a success. Our results showed clear cut differences in renal function between the various control, normothermic bypass, and hypothermic bypass, periods. We had, therefore, I felt, achieved a satisfactory overall experimental design. It remained to establish the reproducibility of our findings in a sufficiently large number of dogs, before embarking on a final analysis and interpretation, of results.

In future experiments, we would cannulate one ureter only, and that through a small flank incision. Cooling would be induced slowly, with 'step-wise' reduction in body temperature.

Before embarking on this program, however, we felt it wise to confirm the effects of normothermic bypass on renal function. One wondered, too, just how far the oesophageal temperature would drop on bypass alone, with no attempt made at maintaining a body temperature of 37°, as was done here.

- EXPERIMENTAL DATA ON DOG B -

WEIGHT: 20.9 kg.

INFUSIONS: (1) 5% dextrose-water.

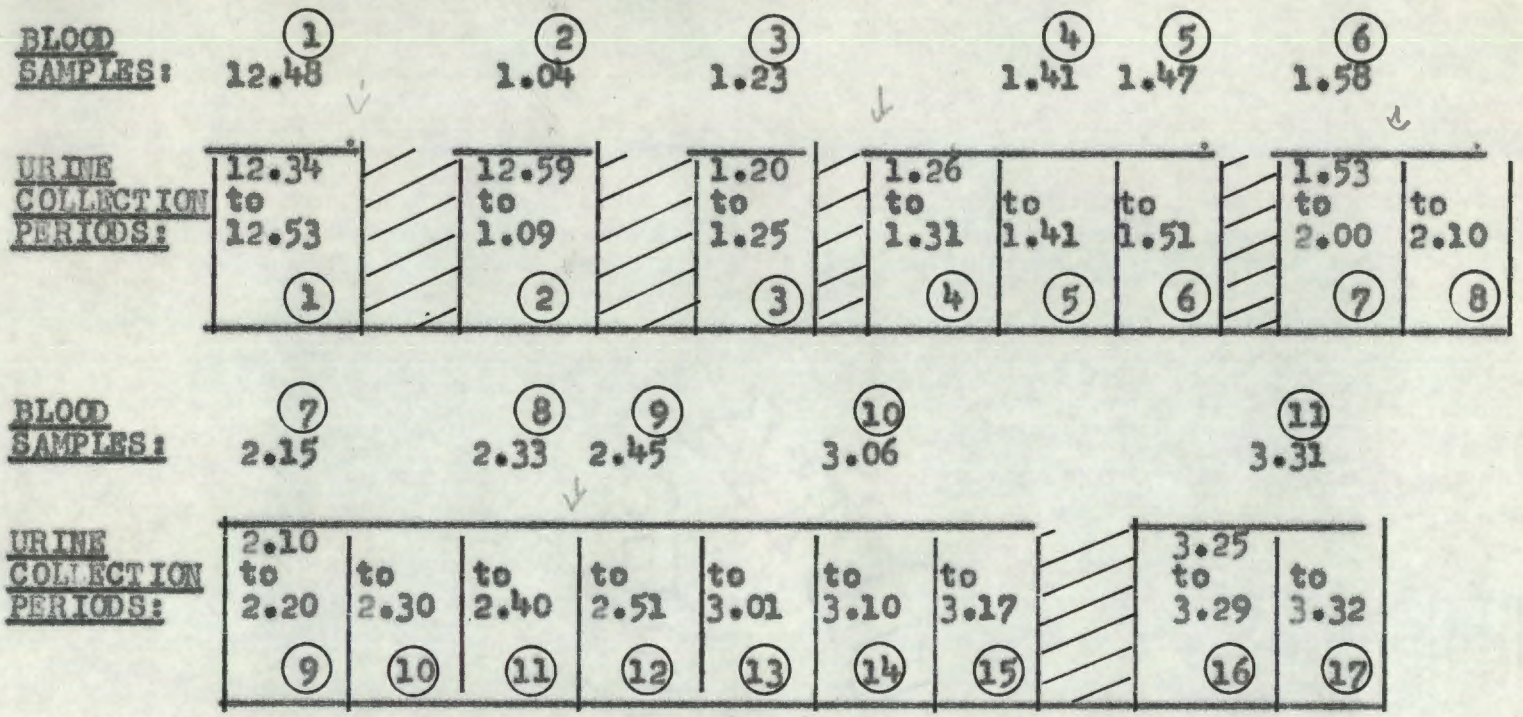
(2) 1.5G creatinine and 2.4G para-amino-hippurate in 'normal' saline, run in at about ¼ ml./minute. Begun at 12.03 p.m.

PRIMING DOSE: of creatinine : 0.5G; of PAH, 1.8G. Given at 12.11 p.m.

EQUILIBRATION PERIOD: 32 minutes.

URINE COLLECTION via polyethylene catheters inserted into ureteral orifices via bladder, (bladder opened through transperitoneal, anterior abdominal, midline incision).

TIMES OF BLOOD AND URINE COLLECTIONS:



As in Dog A, circled figures refer to sample numbers; the other figures refer to times of collection. Figures pertaining to blood samples are given above the continuous series of squares; the latter represent consecutive urine collections. Shaded areas represent time intervals during which urine was not collected.

Cardiopulmonary bypass was initiated at 12.55 pm, blood stream cooling at 1.26 pm, rewarming at 2.00 pm, and Bypass terminated, with resumption of normal circulation at 2.40 pm.

Stop-flow experiments were attempted between periods 2-3, 6-7, and 15-16, by first occluding the ureteral catheters for a few minutes, and then collecting the ensueing urine in a series of small tubes. The attempts between periods 2-3 and 6-7 were unsuccessful as the urinary flow rate at these times was too slow. Such urine as was collected was discarded.

OBSERVED DATA:

URINE

Urine No.	1	2	3	4	5	6	7	8	9	10
Volume (ml)	30.0	19.2	5.0	21.7	16.2	14.1	13.0	12.6	18.0	8.8
Osmolality (mOsm/L)	469	480	601	460	361	334	321	353	444	445
Creatinine (mg./100 ml)	107.3	70.9	131.8	93.6	30.5	15.5	18.3	30.9	113.6	122.8
Na (meq./L)	117.5	38.2	4.25	46.0	105.6	132.4	134.4	88.0	5.0	5.6
K (meq./L)	20.0	14.0	16.3	14.5	6.8	3.0	2.7	11.1	14.9	12.4
Ca (mg./100 ml)	-	-	-	5.95	5.64	3.72	4.47	7.04	-	0.78
Mg (mg./100 ml)	-	2.87	-	1.89	2.36	1.84	1.46	1.06	0.15	-
* PAH (mg./100 ml)	10.55	5.55	7.28	6.09	1.97	0.90	0.56	1.84	4.44	5.31
‡ Protein (mg./100 ml)	tr	tr	-	tr	o	o	o	tr	10	15
Inorganic Phosphate (mg./100 ml)	2.56	1.16	1.18	-	1.50	1.28	0.85	0.85	0.43	0.64

URINE (Continued)

Urine No.	11	12	13	14	15	16	17
Volume (ml)	21.3	31.3	38.4	54.4	64.4	46.0	45.9
Osmolality (mOsm/L)	401	319	250	177	192	189	182
Creatinine (mg/100 ml)	163.6	70.0	54.5	40.9	44.1	27.3	31.4
Na (meq./L)	25.0	41.8	32.2	28.6	53.4	54.4	44.8
K (meq./L)	15.3	7.5	5.5	3.1	3.3	1.9	2.2
Ca (mg./100 ml)	1.33	2.56	2.22	2.22	4.78	5.45	-
Mg (mg./100 ml)	0.69	0.36	0.0	0.74	0.82	0.78	0.82
* PAH (mg/100 ml)	9.19	4.58	3.22	2.29	2.35	-	1.38
ǂ Protein (mg/100 ml)	35	tr	tr	tr	tr	tr	0
Inorganic Phosphate (Mg./100 ml)	0.86	0.83	-	-	-	0.61	0.56

ǂ Protein is measured to the nearest 10 mgm/100 ml. tr. = trace, and is less than 10 mg/100 ml.

* Urine was diluted X250 for PAH estimations; the values given here are not corrected for this dilution.

BLOOD:

Blood Sample	1	2	3	4	5	6	7	8	9	10	11
Osmolality (mOsm/L)	298	-	-	-	303	-	-	317	315	-	-
Creatinine (mg./100 ml)	3.09	2.73	3.82	4.00	4.36	5.00	4.91	5.00	5.82	5.91	5.55
Na (meq./litre)	143.5	-	137.5	139.5	142.5	139.5	138.0	143.0	-	145.0	145.0
K (meq./L)	2.85	2.85	2.50	2.43	2.20	2.33	2.43	2.24	2.33	2.33	2.43
Ca (mg/100 ml)	-	8.82	-	8.44	-	6.36	-	7.40	7.24	-	7.66
Mg (mg/100 ml)	1.62	-	1.62	-	-	1.24	1.39	-	-	-	1.35
PAH (mg/100 ml)	46.8	34.2	43.3	-	59.5	-	-	65.5	72.8	72.4	60.0
Inorganic Phosphate mg/100 ml	-	-	3.10	3.80	3.60	3.20	3.10	-	2.30	-	-

Plasma haemoglobin immediately after conclusion of experiment:

54 mg/100 ml.

Time (P.M.)	Oesophageal Temperature °C	Arterial Blood Pressure mm Hg.
12.50	33.6	90
.55	-	110
1.00	33.8	110
.05	35.5	110
.10	36.6	110
.15	36.9	110
.17	37.0	
.20	37.0	110
.25	36.4	
1.27	35.8	110
.30	30.1	110
.32	26.5	90
.35	23.6	
.42		65
.43	13.8	65
.46	11.4	65
.48	10.4	
.50	9.9	
.52	9.3	
.54	10.4	
.56	11.9	
.58	13.3	58
2.00	14.0	

Time (P.M.)	Oesophageal Temperature °C	Arterial Blood Pressure mm Hg.
2.01	16.3	
.04	24.3	
.05	27.5	
.07	29.6	70
.10	31.9	
.12	34.6	60
.15	36.1	
.17	36.2	
.19	36.4	60
.22	37.4	
.24	38.1	
.26	38.4	
.28	38.3	
.30	38.6	
.32	38.3	55
.36	38.1	70
.38	36.2	
.41	36.0	80
.45	35.5	80
.50	34.1	

45 ml. $NaHCO_3$ given I.V. at 2.12 p.m.

- DERIVED DATA -

PERIOD	1	2	3	4	5	6	7	8
Volume (ml/min)	3.00	1.92	1.00	4.32	1.62	1.41	1.86	1.26
Midperiod Serum:								
Creatinine (mg/100 ml)	3.09	2.73	3.79	3.88	3.95	4.30	4.93	4.96
Na (meq/l)	143.5	140.8	137.6	138.7	138.9	142.0	139.9	138.9
K(meq/l)	2.85	2.85	2.51	2.48	2.45	2.24	2.31	2.37
Ca (mg./100 ml)	-	8.82	8.63	8.57	8.49	7.83	6.54	6.57
Mg (mg/100 ml)	1.62	1.62	1.62	1.56	1.48	1.37	1.26	1.30
Inorganic Phosphate (mg/100 ml)	-	-	-	3.31	3.61	3.63	3.25	3.16
PAH (mg/100 ml)	46.8	34.2	43.1	47.0	52.1	58.8	60.7	61.9
Average Temperature °C	-	35.1	36.7	32.6	22.7	11.9	12.1	25.5
Ccr (ml./min)	104.2	49.8	34.7	104.2	12.5	5.08	6.90	7.85
CPAH (mg/min)	170.5	78.0	42.3	139.5	15.3	5.39	4.3	9.4
Tm PAH (mg/min)	34.2	11.1	4.4	20.8	2.0	0.4	-0.7	1.3
% Excretion of filtered:								
Na	2.4	1.1	0.1	1.4	9.9	25.9	25.9	10.2
K	20.2	18.9	18.7	24.2	36.0	37.2	31.5	51.0
Ca	-	-	-	2.9	8.6	13.2	18.4	17.2
Mg	-	6.8	-	5.0	20.7	37.2	31.3	13.1
P	-	-	-	-	5.4	9.8	7.1	4.3
H_2O	2.9	3.9	2.9	4.2	13.0	27.8	27.0	16.1

PERIOD	9	10	11	12	13	14	15	16	17
Volume (ml/min)	1.80	0.88	2.13	2.85	3.84	6.04	9.20	11.50	15.30
Midperiod Serum:									
Creatinine (mg/100 ml)	4.91	4.96	5.13	5.82	5.87	5.91	5.80	5.59	5.55
Na (meq/l)	138.0	140.8	143.1	143.8	144.4	145.0	145.0	145.0	145.0
K (meq/l)	2.43								
Ca (mg/100 ml)	6.87	7.16	7.37	7.24	7.31	7.39	7.45	7.56	-
Mg (mg/100 ml)	1.39	1.38	1.38	1.37	1.37	1.36	1.36	1.35	1.35
P (mg/100 ml)	3.10	2.83	2.57	2.30	-	-	-	-	-
PAH (mg/100 ml)	63.2	64.5	66.6	72.8	72.4	72.4	68.7	61.9	60.1
Average Temperature °C	35.4	37.7	37.6	35.3	-	-	9	-	-
Ccr (ml/minute)	41.7	21.8	68.0	34.3	35.7	41.8	70.0	56.2	86.6
C_{PAH} (ml/minute)	31.7	18.1	73.4	44.8	42.7	47.8	78.6	-	87.9
TmPAH (mg./minute)	-4.3	-2.7	2.5	9.7	7.1	6.7	9.9	-	5.0
% Excretion of filtered									
Na	1.6	0.2	0.6	2.4	2.4	2.9	4.8	7.7	5.5
K	26.5	21.6	21.3	26.8	25.4	19.2	18.4	16.1	16.0
Ca	-	0.4	0.6	2.9	3.3	4.3	8.4	14.7	-
Mg	4.7	-	1.6	2.2	0.0	7.9	7.9	11.8	10.7
P	0.6	0.9	1.0	0.03	-	-	-	-	-
H_2O	4.3	4.0	3.1	8.3	10.8	14.5	13.1	20.5	17.7

CHAPTER V.

NORMOTHERMIC BYPASS.

Dog B. had shown that cardiopulmonary bypass, at normal body temperature, could have profound effects on renal function. We now wished to confirm these findings, and study also renal function in the immediate post-normothermic bypass period. This seemed essential if we were to later correctly evaluate the effects produced by hypothermia on postoperative renal function.

In order to keep Dog B at an oesophageal temperature of 37°, it had been necessary to actively warm the blood passing through the extracorporeal circuit. This meant more labour for the technician in charge of the heat-exchange unit. It seemed of interest therefore to determine just how big the drop in oesophageal temperature would be, and how deranged the renal function were bypass to be performed <u>without</u> using the heat exchange unit.

NORMOTHERMIC BYPASS - DOG 1.

The protocol for this experiment was simply to collect blood and urine samples throughout half an hour of normothermic bypass, as well as during short control,

pre and post-bypass periods.

The experimental procedure was almost identical to that used in Dog B, with two exceptions. Cooling was not induced at any stage. Urine was collected via a short polyethylene catheter placed in the right ureter. (This had been exposed 2 days previously through a small flank incision and dissected out retroperitoneally). The catheter tip was placed well within the renal pelvis.

RESULTS:

The results are shown graphically in Figs. 5 and 6. Details are given at the end of this Chapter.

URINE FLOW RATE, OSMOLALITY, Ccr, AND TmPAH (FIG. 5)

In the 55 minutes of observation before bypass was begun, the urine flow rate fell, and urinary osmolality rose. The Ccr fell slightly, from 21.6 ml/minute to 18.4 ml/minute. The arterial systolic blood pressure was maintained at 130 mm.Hg. until 20 minutes before bypass, when it fell to 90 mm.Hg. This fall in blood pressure was not accompanied by any alteration in urine flow rate or osmolality, but the Ccr fell further, from 18.4 ml/minute to 14.3 ml/minute.

Bypass exaggerated prevailing trends, so that urine flow rate and Ccr dropped sharply; urinary osmolality rose.

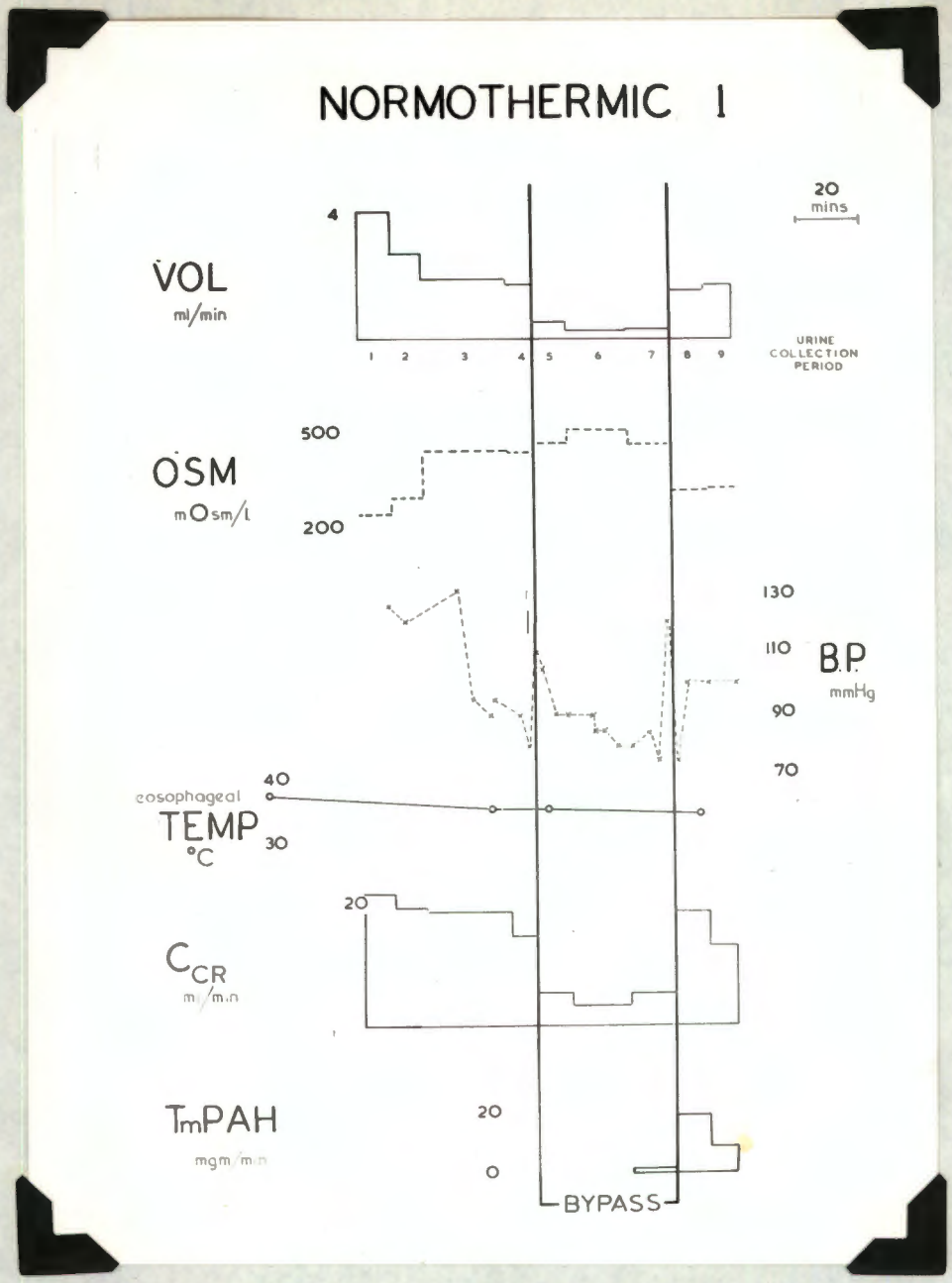

NORMOTHERMIC 1
20 mins
VOL
ml/min
4
1 2 3 4 5 6 7 8 9
URINE COLLECTION PERIOD
OSM
mOsm/l
500
200
130
110
90
70
B.P
mmHg
oesophageal
TEMP
°C
40
30
20
C_{CR}
ml/min
T_mPAH
mgm/min
20
0
BYPASS

FIG. 5.

The arterial blood pressure, after an initial peak just at and immediately after the initiation of bypass, stayed close to its pre-bypass level of about 90 mm Hg.

Discontinuation of bypass lead to a reversal of the above. Urine flow rate and Ccr rose immediately, while urinary osmolality fell, to levels comparable to those prevailing immediately before bypass.

The calculated TmPAH varied irregularly, and at low levels, both before and during bypass. On resumption of normal circulation, the TmPAH rose sharply, and momentarily to levels far higher than before.

URINARY ELECTROLYTE AND PAH CONCENTRATION CHANGES. (FIG. 6)

All these substances showed a progressive rise in urinary concentration during the initial control periods 1 to 4. Their behaviour differed markedly thereafter however.

During bypass, urinary Na and Mg concentrations fell rapidly, to very low levels. Thus by period 7, after 30 minutes of bypass, urinary Na concentration was but 3 meq/L., while Mg. was undetectable. Both elements returned to higher concentrations in the post-bypass periods.

The urinary Ca and inorganic phosphate concentrations rose transitorily on initiation of bypass, only to fall

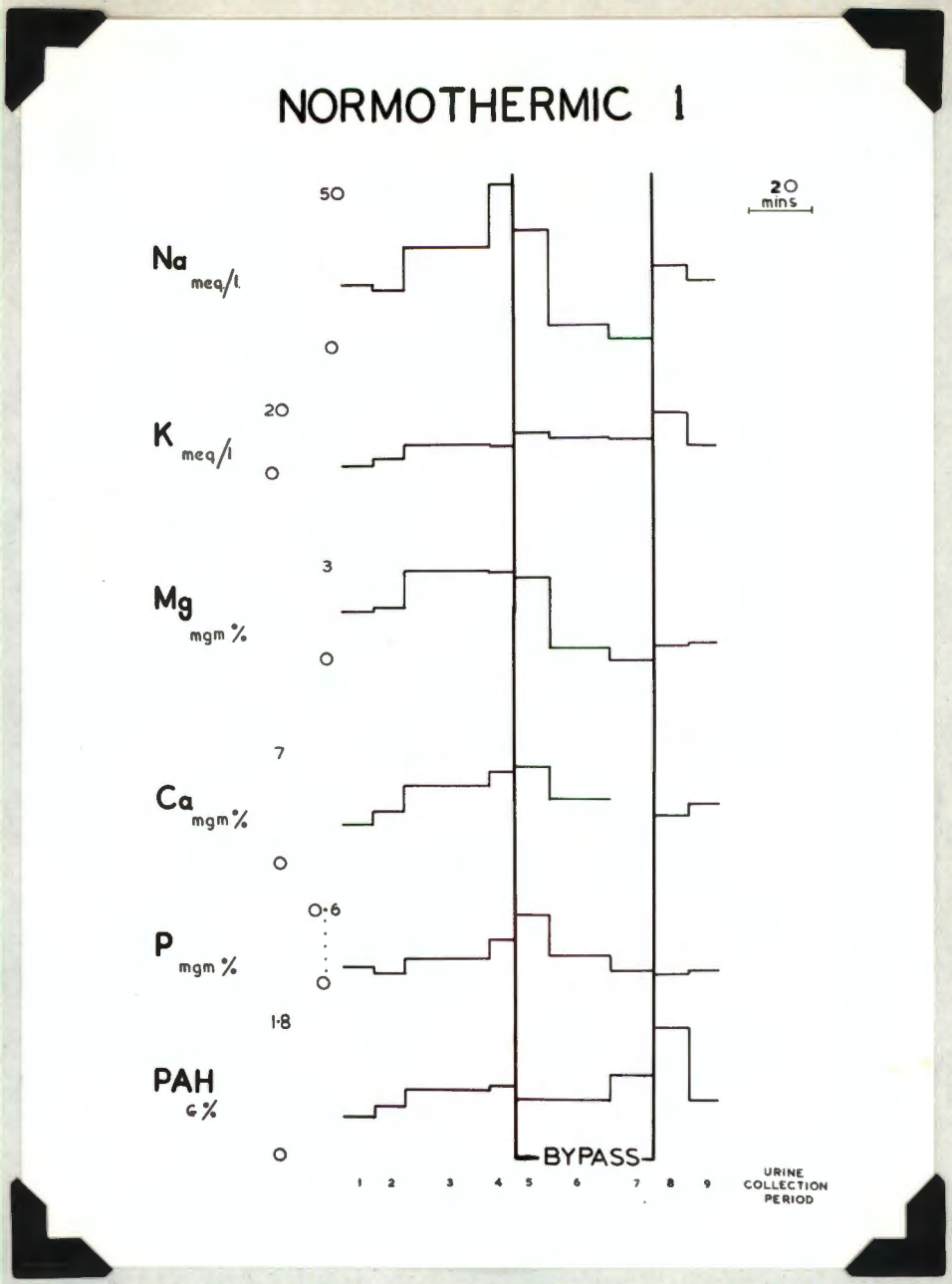

NORMOTHERMIC 1
20 mins
Na meq/l
50
0
K meq/l
20
0
Mg mgm %
3
0
Ca mgm %
7
0
P mgm %
0·6
0
PAH c %
1·8
0
BYPASS
1 2 3 4 5 6 7 8 9
URINE COLLECTION PERIOD

FIG. 6.

thereafter. The calcium concentration of urine sample 7 was not determined; urine inorganic phosphate concentration never fell to levels lower than those pertaining during the control pre-bypass periods. After resumption of normal circulation , (periods 8 and 9) both Ca and inorganic phosphate remained at low levels.

The urinary K concentration attained slightly higher levels during bypass, than previously. After discontinuation of bypass, it rose even higher.

Initiation of bypass was accompanied by a slight fall in urinary PAH concentration. This rose during period 7, rose again immediately post-bypass, and finally fell during period 9.

PERCENTAGE EXCRETION OF FILTERED ELECTROLYTES AND WATER:

The percentage excretion of Na and Mg fell sharply during bypass, only to regain control values thereafter. Thus Na fell from 2.0 - 4.8% to 0.2%, and returned to 1.7 and 2.2%. Mg fell from control levels of 14.0% - 22.9%, to 0.0%; immediately after discontinuation of bypass, this rose to 23.0%, then fell to 5.0%.

Water paralled the behaviour of Na and Mg. falling from 10.6% - 19.4%, to 7.1%, by the end of the bypass period.

On resumption of normal circulation, this rose to 8.6% and 13.9% .

The percentage excretion of filtered K, rose from the wide control range of 18.6% - 39.0%, to 48.4% on initiation of bypass. It then fell to 29.6%. After cessation of bypass, it rose to 63.7% and 51.8% in periods 8 and 9.

Ca and inorganic phosphate showed minor variations in percentage excretion throughout the experiment. These suggest a rise in excretion during periods 1 to 4, a slight fall during bypass, and a minor rise thereafter. The variations seem too small, however, to be significant.

URINE PROTEIN CONCENTRATION:

A trace of protein appeared in the urine, during period 6, eleven minutes after commencement of bypass. This was present for the remainder of the experiment.

SERUM CONCENTRATION CHANGES:

Serum osmolality fell from 306 mOsm./l to 290 mOsm/l during the initial control period. During bypass this rose abruptly to 314 mOsm/l and 310 mOsm/l. At the conclusion of the experiment, serum osmolality was 320 mOsm/l.

Creatinine concentration rose from 6.37 mg/100 ml to 7.82 mg/100 ml pre-bypass, fell during bypass to 6.37 mg/100 ml and rose again thereafter to 7.18 mg/100 ml. Creatinine was not determined on blood sample 4, taken 11 minutes after commencement of bypass.

Serum Na rose from 133 meq./l to 139 meq/l in blood 4, then fell to 130 meq/l during bypass. After resumption of normal circulation it reached 134.5 meq/l.

Serum K concentration showed minor fluctuations throughout the experiment, ranging between 2.62 meq/l and 3.00 meq/l. The concentrations in bloods 5, 6 and 8 (7 not done) were lower than those in blood samples 1 to 4.

Serum calcium concentration was measured in only 4 of the 8 blood samples. The single sample taken during the bypass period was 9.2 mg/100 ml, in contrast to 10.2 mg/100 ml and 10.5 mg/100 ml before, and 10.1 mg/100 ml after bypass.

Mg showed no significant alteration throughout the experiment, varying irregularly between 1.56 and 1.80 mg/100 ml.

The serum inorganic phosphate fell progressively, from 4.54 initially, to 2.97 mg/100 ml in serum sample 7.

PAH concentration fell sharply in serum samples 1 to 3, from 111.9 mg/100 ml to 76.5 mg/100 ml. In samples 5 to 8, it fluctuated between 60.6 mg/100 ml and 67.1 mg/100 ml. PAH was not determined in serum sample 4, the first blood sample taken after commencement of bypass.

COMMENT:

The most striking technical inadequacy of this experiment is found in the 'control' periods 1 to 4, during which time bloods 1 to 3 were drawn. The urinary flow rate fell from 4.15 ml/min. to 1.79 ml/minute, while urinary electrolyte, PAH and osmolar concentrations all increased. While these findings may be partly due to the concomitant fall in GFR (as revealed by the Ccr) the fall in serum PAH concentrations (from 111.9 mg/100 ml to 76.5 mg/100 ml) suggests that the rate of the intravenous infusions had diminished. This suspicion is strengthened on noting the fall in serum osmolality over the same period, from 306 mOsm/l to 290 mOsm/l. It was pointed out in Chapter III that the intravenous infusion of hypertonic 10% Dextrose water tends to raise the serum osmolality, a phenomenon that I have repeatedly observed in other work; we have already encountered it here in Dog B, and it will be seen again in later Chapters. This suspicion of a slowing drip rate hardens into certainty when one notes that the fall

in serum PAH concentration occurs in the face of a decreasing urinary loss, before the introduction of unprimed blood from the extracorporeal circuit, and after an equilibration period of 42 minutes.

On the other hand, it will be recalled that the vehicle for the sustaining infusion of PAH and Creatinine consisted of N saline, while the 10% dextrose water infusion was in a separate vacolitre, fed through a separate drip set into a vein in another limb. One would have to postulate that both drips slowed concurrently, an explanation that does not account for the observation that serum creatinine actually rose during this same period, (from 6.37 mg/100 ml to 7.82 mg/100 ml).

While it is clear that the marked fall in plasma PAH concentrations during control periods 1 to 4, renders all calculations on renal handling of PAH during this time, highly suspect, it is of interest nevertheless to consider the variations of calculated C_{PAH} throughout the experiment (cf details at end of Chapter). During the first two periods, C_{PAH} is actually slightly lower than the Ccr. An exceedingly high plasma PAH concentration will cause the C_{PAH} to approach f.Ccr. (where f = filterable fraction of PAH). (Ref. 159). The plasma PAH concentration here however is only twice the order of concentration usual

in such experiments. Other factors that may be operating to reduce C_{PAH} are a fall in E, the extraction ratio of PAH, and more particularly, self depression of TmPAH i.e. inhibition or even complete paralysis of tubular transport of PAH. In periods 3 and 4, plasma PAH concentration falls and C_{PAH} rises slightly. In periods 6 and 7, C_{PAH} is again almost identical with Ccr, although the plasma PAH concentration is now at conventional levels. There seems to be no other explanation for this observation than that postulated above, i.e. self depression of TmPAH. Post-bypass, CPAH rises remarkably, although still not to its usual normal value of approximately three times the Ccr. (cf Appendix C). But this of course is only to be expected; when performing Tm experiments, C_{PAH} is always depressed, as E must of necessity be depressed too.

How valid are the TmPAH calculations? An essential perquisite of such measurements is that the load of PAH delivered to the tubules be substantially greater than the quantity excreted by them; Smith (159) demands a load/tubular excretion ratio of more than 1.5; Load/Tubular Excretion ratio is $= \frac{\overline{C}PAH - f.Ccr}{CPAH - f.Ccr}$

where $\overline{C}PAH$ is the PAH clearance at low plasma PAH concentration, i.e. ERPF.

The actual value for $\overline{C}_{PAH}$ in this experiment is unknown. The Cer, at 2.67 ml/kg/minute in period 1, and 1.59 ml/kg/minute in period 9, is within normal limits, as given by Asheim et al, and Kolberg (cf Appendix C). If we assume $\overline{C}_{PAH}$ to be the usual normal value of $\pm$ three times the Cer, and substitute in the above equation (for period 9), then:

$$\text{Load/Tubular Excretion ratio} = \frac{40 - (.92 \times 12.8)}{23.7 - (.92 \times 12.8)}$$

$$= 2.37$$

well above that demanded for evidence of tubular saturation.

As pointed out above, the great variation in plasma PAH concentration during periods 1 to 4 render calculations here most uncertain, and in any event, I have already postulated tubular paralysis for PAH transport at this time. This applies to the bypass period too. Post-bypass however the figures for TmPAH may be meaningful. The plasma PAH concentration is now relatively constant, and the C_{PAH} behaving as expected.

To sum up, all the calculations given here, relating to PAH transport, are suspect, and those dealing with the initial control and bypass periods are vitiated by the appearance of the phenomenon of self depression of TmPAH. Probably only the figures for TmPAH in the last two periods, 8 and 9, are meaningful. It is for

these reasons that TmPAH is plotted for only the last 3 periods, in Fig. 5. If the TmPAH's in periods 8 and 9 are corrected for temperature (10% increase for 1° fall below 37°, as recommended by Smith (159)), and doubled - the actual figures given are derived from one kidney only - they then fall well within the normal range for TmPAH.

The expected fall in plasma concentrations of Cr and PAH after introduction of bypass, may or may not be present. In the absence of Cr and PAH estimations on blood No. 4, it is impossible to decide this point.

EXPERIMENTAL DATA ON NORMOTHERMIC BYPASS DOG 1.

Weight: 16.1 Kg

Infusions: 1. 10% Dextrose-water

2. Creatinine 1.5 G and PAH 2.4 G in N. saline

Priming Dose of creatinine 0.5 G; of PAH 1.8 G; given at 11.03 a.m.

Equilibration period: 42 minutes

Urine collection via short polyethylene catheter tied into R ureter; catheter tip within renal pelvis.

Times of Blood and Urine collections:

Blood Samples:	① 11.55	② 12.03	③ 12.35	④ 12.51	⑤ 1.02	⑥ 1.19	⑦ 1.28	⑧ 1.40
Urine collection periods:	11.45 to 11.55 ①	to 12.05 ②	to 12.32 ③	to 12.40 ④	to 12.51 ⑤	to 1.10 ⑥	to 1.24 ⑦	to 1.35 ⑧	to 1.44 p.m. ⑨

Cardiopulmonary bypass began at 12.40; discontinued at 1.24.

Oesophageal temperature kept at 37.2° to 34° throughout.

TIME	OESOPHAGEAL TEMPERATURE °C	ARTERIAL BLOOD PRESSURE mm Hg.
11.15	37.2°	
.54		125
.59		120
12.15		130
.20		95
.26	35.0	90
.27		95
.35		90
.38		80
12.40		110
.42		105
.44	35.0	
.46		90
.50		90

TIME	OESOPHAGEAL TEMPERATURE °C	ARTERIAL BLOOD PRESSURE mm Hg.
12.58		90
.59		85
1.02		85
.06		80
.11		80
1.16		85
1.19		75
1.22		120
1.25		75
1.28		100
1.32	34°	
1.35		100
1.44		100

OBSERVED DATA.

URINE

Urine No.	1	2	3	4	5	6	7	8	9
Volume (ml)	41.5	28.0	52.5	14.3	6.5	5.5	5.0	17.5	16.0
Osmolality (mOsm/L)	244	299	445	442	469	510	465	320	323
Creatinine (mg/100 ml)	33.5	45.5	68.7	62.0	67.2	76.8	91.0	83.6	51.5
Na (meq/L)	20.5	18.4	32.2	52.8	38.0	7.2	3.0	26.1	21.5
K (meq/L)	2.8	5.2	9.5	9.0	12.8	11.2	11.1	19.6	9.9
Ca (mg/100 ml)	2.52	3.36	4.97	5.91	6.23	4.22	-	3.17	3.90
Mg (mg/100 ml)	1.59	1.69	2.93	2.88	2.62	0.42	0.00	0.46	0.59
PAH (G/100 ml)	.552	.683	.928	.986	.808	.808	1.150	1.782	.811
‡ Protein (mg/100 ml)	o	o	o	o	o	tr	tr	tr	tr
Inorganic Phosphate (mg/100 ml)	0.139	.087	.212	.365	.557	.244	.122	.087	.122

‡ Protein is measured to the nearest 10 mg/100 ml.

BLOOD

Blood Sample	1	2	3	4	5	6	7	8
Osmolality (mOsm./L)	306	300	290	314	-	310	-	320
Creatinine (mg/100 ml)	6.37	6.78	7.82	-	6.85	6.37	7.18	7.18
Na (meq/L)	133	137.5	-	139	132	130	134	134.5
K (meq/L)	2.88	2.75	2.88	3.00	2.62	2.65	-	2.63
Ca (mg/100 ml)	10.2	-	10.5	-	9.2	-	10.1	-
Mg (mg/100 ml)	1.71	1.80	1.56	1.72	1.73	1.79	-	1.65
PAH (mg/100 ml)	111.9	108.4	76.5	-	61.0	67.1	65.5	60.6
Inorganic Phosphate (mg/100 ml)	4.54	4.48	4.10	3.86	3.48	-	2.97	-

Plasma Haemoglobin at conclusion of experiment : 35 mg/100 ml.

DERIVED DATA.

PERIOD	1 ≠	2	3	4	5	6	7	8	9
Urine Volume (ml/min.)	4.15	2.80	1.95	1.79	0.59	0.29	0.36	1.59	1.78
Midperiod Serum:									
Creatinine (mg/100 ml)	6.37	6.63	7.28	7.78	7.44	6.90	6.43	7.18	7.18
Na (meq/L)	133.0	135.8	138.0	138.6	138.8	132.9	130.2	134.1	134.5
K (meq/L)	2.88	2.80	2.81	2.89	2.96	2.67	2.65	2.65	2.65
Ca (mg/100 ml)	10.2	10.2	10.4	10.5	10.0	9.3	9.7	-	-
Mg (mg/100 ml)	1.71	1.76	1.68	1.57	1.67	1.73	1.78	1.72	1.65
Inorganic Phosphate (mg/100 ml)	4.54	4.48	4.28	4.08	3.94	3.53	3.19	-	-
PAH (mg/100 ml)	111.19	109.7	92.9	76.5	-	61.9	66.4	64.9	60.6
Average Temperature °C			35.0					34.0	
Ccr (ml/min)	21.6	19.2	18.4	14.3	5.3	3.2	5.1	18.5	12.8
CPAH (ml/min)	19.4	17.4	19.6	23.2	-	3.8	6.3	46.0	23.7
TmPAH (mg/min)	0.7	-.2	2.7	7.6	-	5.2	1.0	17.4	7.4
% Excretion of filtered:									
Na	3.0	1.0	2.5	4.8	3.1	0.5	0.2	1.7	2.2
K	18.6	27.2	35.8	39.0	48.4	38.6	29.6	63.7	51.8
Ca	4.8	4.8	5.1	7.1	6.9	4.1	-	2.7 *	5.4 *
Mg	17.9	14.0	18.5	22.9	17.5	2.2	0.0	23.0	5.0
P	0.6	0.3	0.5	1.1	1.6	0.6	0.3	0.3 *	0.6 *
H_2O	19.4	14.6	10.6	12.5	11.1	9.1	7.1	8.6	13.9

≠ The first blood sample was withdrawn 5 minutes after the midpoint of period 1. All the figures pertaining to period 1 must therefore be regarded as approximations.

* Serum concentrations of Ca and inorganic phosphate were not determined on blood sample No. 8. The percentages given assume their concentrations at the midpoint of periods 8 and 9, were equal to those of blood sample 7.

'NORMOTHERMIC' DOG 2.

The aim of this experiment was to ascertain the degree of cooling, and renal malfunction produced by cardiopulmonary bypass alone, without the use of the heat exchanger. The experimental procedure adopted was otherwise similar to that for Normothermic Dog 1. PAH was not used.

RESULTS:

These are presented graphically in Figs. 7 and 8. Details are given at the end of this chapter.

URINARY FLOW RATE, OSMOLALITY, Ccr, AND ARTERIAL SYSTOLIC BLOOD PRESSURE. (FIG. 7):

Control observations were made over an hour, prior to bypass. Both urine flow rate and Ccr dropped during this time. Urinary osmolality rose, from 224 mOsm./l., to 410 mOsm./l. The arterial blood pressure was recorded for the first 13 minutes only; the next reading was taken 5 minutes after bypass was commenced. Such readings as there are vary between 136 mm Hg. and 145 mm Hg. The oesophageal temperature dropped from 35.6° to 32.8°.

Three urine samples were collected during the 34 minutes of bypass. During the first such, the urine flow rate remained unchanged from that obtaining immediately pre-bypass; the Ccr rose from 30.6 ml/min.

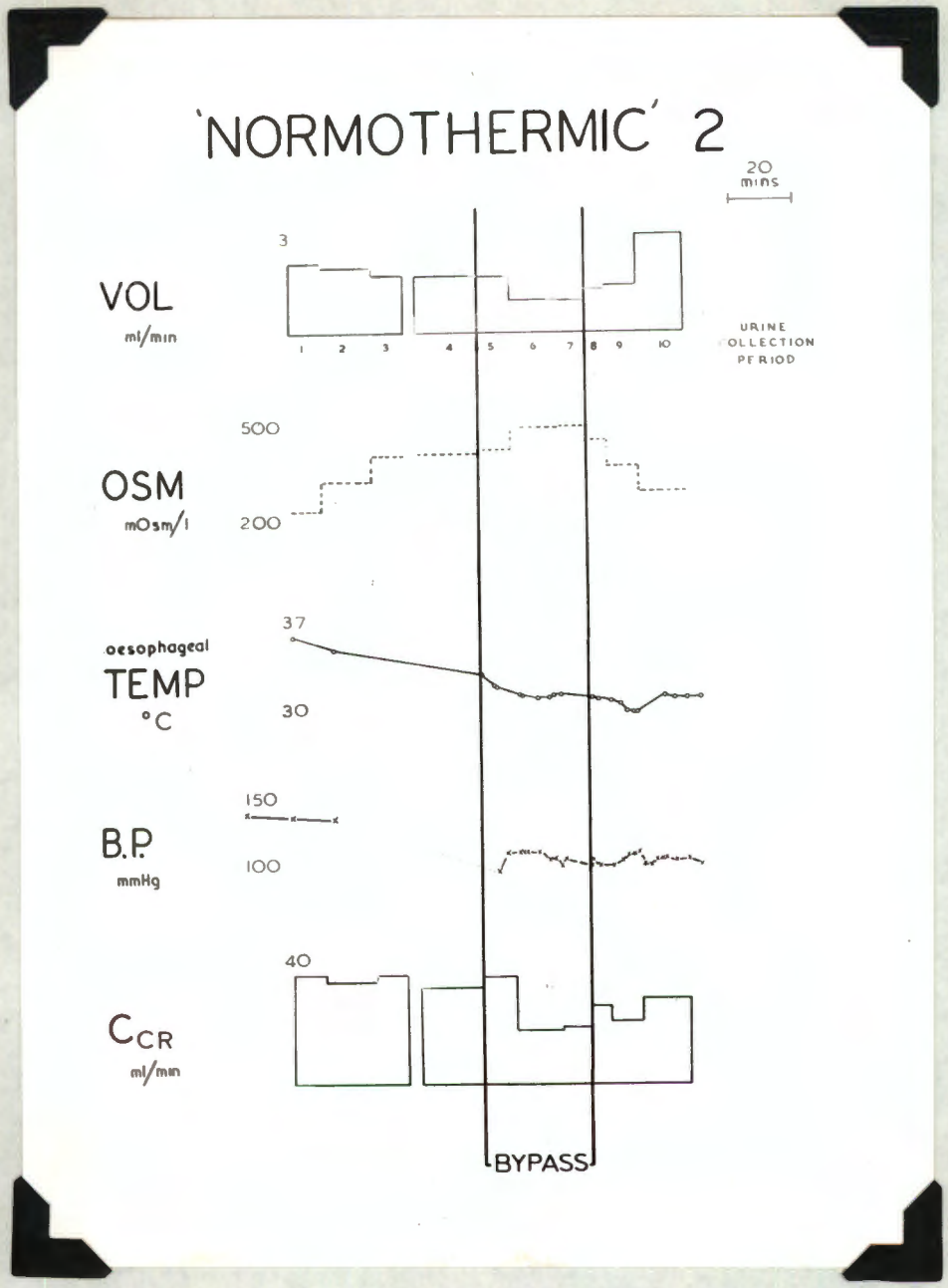

'NORMOTHERMIC' 2
20 mins
VOL
ml/min
3
1 2 3 4 5 6 7 8 9 10
URINE COLLECTION PERIOD
OSM
mOsm/l
500
200
oesophageal
TEMP
°C
37
30
B.P.
mmHg
150
100
CCR
ml/min
40
BYPASS

FIG. 7.

to 34.9 ml/minute. Urinary osmolality rose, fractionally. By the end of the bypass period, urinary flow rate was noticeably reduced, as was the Ccr. Urinary osmolality rose to 495 mOsm./l. The oesophageal temperature dropped abruptly from 32.8° to a low of 30.9°. The arterial blood pressure rose from 95 mm Hg. (5 minutes after commencement of bypass) to 110 mm Hg., and then gradually subsided to 100 mm Hg.

The post bypass period witnessed a reversal of the above. Immediately bypass was discontinued, urine flow rate rose, to levels above those present before bypass. This diuresis was accompanied by a fall in urine osmolality. The Ccr rose, irregularly, but did not regain its initial level.

URINARY ELECTROLYTE CONCENTRATION CHANGES. (FIG. 8)

During periods 1 to 3, urinary Na, K, Mg, Ca and inorganic phosphate concentrations varied but slightly. The largest single changes seen pre-bypass were those between periods 3 and 4; urine Na concentration rose from 69.8 meq./l to 97.6 meq./l., Mg. concentration fell from 3.28 mg./100 ml. to 2.33 mg./100 ml., Ca rose from 6.1 mg/100 ml to 7.4 mg./100 ml, and inorganic phosphate fell from 0.98 mg./100 ml. to 0.80 mg./100 ml. The urinary K concentration fell from 14.4 meq./l to 12.0 meq./., but had been at this level during periods 1 and 2.

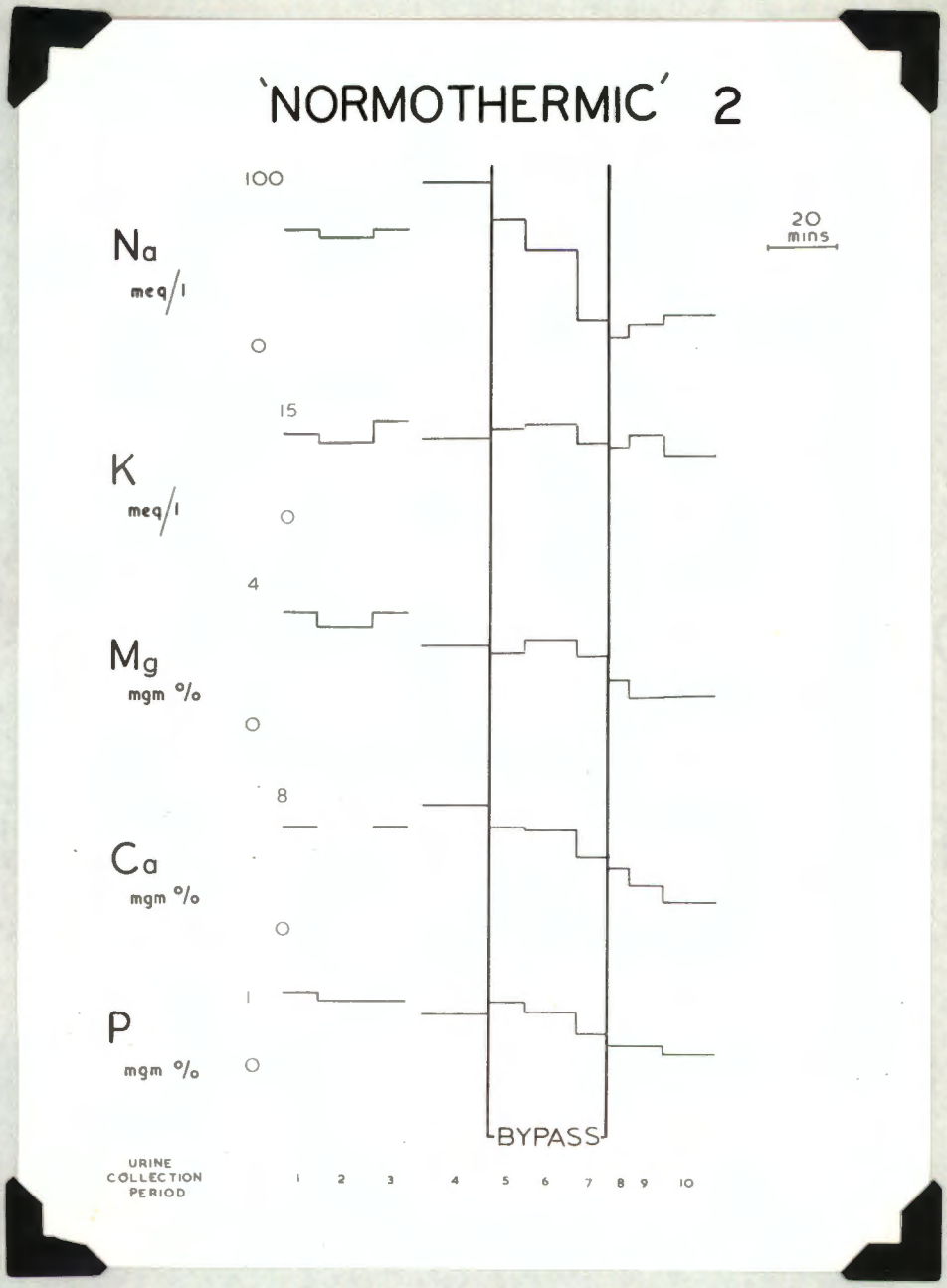

'NORMOTHERMIC' 2
100
Na
meq/l
0
15
K
meq/l
0
4
Mg
mgm %
0
8
Ca
mgm %
0
1
P
mgm %
0
20 mins
BYPASS
URINE COLLECTION PERIOD
1 2 3 4 5 6 7 8 9 10

FIG. 8.

Bypass led to a sharp fall in urine Na concentration, to 17 meq./l. This fell further, to 6.4 meq./l immediately after bypass was discontinued, but then rose to 19.4 meq/l.

The urine K concentration showed no significant change throughout the experiment, varying irregularly between 14.4 meq./l (pre-bypass) to 9.4 meq./l (period 10.)

Mg. concentrations fell from pre-bypass levels of 3.28 - 2.33 mg./100 ml, to post bypass levels of 1.35 - 0.83 mg./100 ml. Bypass produced no change in urine Mg. concentration, from that present in period 4.

The urine Ca concentration rose slightly from 6.0 mg/100 ml. to 7.4 mg/100 ml. during periods 1. to 4. Bypass caused a progressive drop to 4.3 mg/100 ml, a fall that was continued post-bypass to a final concentration of 1.8 mg./100 ml.

The urinary inorganic phosphate concentration fell gradually throughout the experimental period, from an initial 1.09 mg./100 ml. to a final 0.24 mg./100 ml. Bypass possibly checked this trend initially (period 5), but thereafter seemed to be without effect.

PERCENTAGE EXCRETION OF FILTERED ELECTROLYTES AND WATER:

The percentage excretion of filtered Na rose from 3.2%, 3.1% and 2.6%, in periods 1 to 3, to 4.2% in period 4.

It then fell, during bypass, to 0.7%, but rose again to 1.6% during period 10.

There was no significant alteration in percentage excretion of filtered K until periods 9 and 10, when it rose from previous levels of 17.7 - 23.1%, to 37.9% and 46.2% respectively.

The percentage excretion of Ca and Mg both fell irregularly throughout the course of the experiment. In each case the major fall occurred with the introduction of bypass.

Inorganic phosphate showed no change in percentage excretion until period 7, when it fell from 1.3% to 0.8%. It stayed at this level thereafter.

Periods 9 and 10 saw a rise in percentage excretion of filtered water, from a previous range of 6.4 - 5.2%, to 8.5% and 11.4% respectively.

URINE PROTEIN CONCENTRATION:

There was no proteinuria throughout this experiment.

SERUM CONCENTRATION CHANGES:

Of the 7 blood samples, only two, numbers 4 and 5, were taken during the bypass period. Only the creatinine concentration was determined in blood 4, after which it was mislaid.

Serum osmolality rose from 296 mOsm/l to 300 mOsm/l during the course of the experiment.

The serum creatinine concentration fell from 6.81 mg/100 ml. to 6.38 mg/100 ml pre-bypass. It then fell sharply to 4.22 mg/100 ml. in blood 4, taken 20 minutes after the initiation of bypass. Thereafter, the serum creatinine concentration rose gradually, to 5.26 mg/100 ml. in blood 7.

Serum Na concentration dropped from pre-bypass levels of 140.0 meq./l to 138.5 meq./l., to 134.0 meq./l and 135.0 meq./l postbypass.

There was little change in serum K concentration, until the post-bypass period, when it fell abruptly to 2.98 meq./l and 2.32 meq./l., previously, it had varied irregularly between 3.25 and 4.13 meq./l.

Serum calcium, determined on but 4 of the 7 samples, fell from pre-bypass levels of 10.9 mg/100 ml. to 10.1 mg/100 ml. during, and 10.3 mg/100 ml after bypass.

Serum Mg. fell progressively from 1.61 mg./100 ml, to 1.33 mg./100 ml. and 1.34 mg./100 ml post-bypass. The single Mg. determination during bypass (blood 5) was 1.7 mg./100 ml. however.

Serum inorganic phosphate fell from pre-bypass levels of 4.47 - 4.04 mg./100 ml, to 3.60 mg/100 ml. during, and 3.64 - 3.68 mg./100 ml. after, bypass.

COMMENT:

The fall in oesophageal temperature, to 32.8°, before bypass was commenced, was totally unexpected. This occurred during the period of preparative chest surgery, and presumably is a reflection on our surgical technique. The net result is that, instead of examining the cooling effects of bypass, in an animal with normal heat conserving ability, as we had thought to do, we are now presented with the results of bypass, in an already mildly heat depleted dog.

In these circumstances, bypass may well be expected to bring about a lower oesophageal temperature, than would otherwise have been the case. The picture is further complicated by the failure of the dogs temperature to return to normal levels, post-bypass. Inspection of the curve for oesophageal temperature (Fig. 7) throughout the experiment shows that while a slight acceleration in rate of fall of temperature occurred immediately bypass was begun, the overall effect of bypass, on oesophageal temperature, appears to have been negligible. The only permissible conclusion seems to be that bypass in itself,

in a heat depleted dog, probably does not cause the oesophageal temperature to fall much below about 30°C.

The oesophageal temperature, post-bypass, never regained pre-bypass levels (31° versus 32.8°). Strictly speaking, therefore, one is unable even to compare pre- and post-bypass renal function, as a measure of reversibility of renal effects of bypass. The changes in renal function during the course of this experiment, must therefore be interpreted as those occurring during progressive mild cooling, with the superimposition of the effects of cardiopulmonary bypass during urine collection periods 5, 6 and 7.

For the rest, there are but two points worthy of comment. The arterial blood pressure was not recorded for 47 minutes before, and 5 minutes after, the commencement of bypass. This makes it difficult to evaluate Ccr changes during this time in view of the concomitant mild hypothermia. Secondly, the serum creatinine level dropped immediately after introduction of bypass; this behaviour was expected (cf Chapter 11).

EXPERIMENTAL DATA ON 'NORMOTHERMIC' BYPASS DOG 2.

WEIGHT: 19.6 kg.

INFUSIONS: 1. 10% Dextrose Water
2. Creatinine, 1.5 G in 1 litre of N. saline

PRIMED with Creatinine 0.5 G, at 10.00 a.m.

EQUILIBRATION PERIOD: 30 minutes

URINE COLLECTION: via polyethylene catheter tied into R ureter; catheter tip within renal pelvis.

TIMES OF BLOOD AND URINE COLLECTIONS:

BLOOD SAMPLES:	① 10.30	② 10.56	③ 11.16	④ 11.50	⑤ 11.57	⑥ 12.10	⑦ 12.27

Urine Collection Periods:	10.30 to 10.40 ①	to 10.56 ②	to 11.06 ③	11.10 to 11.30 ④	to 11.40 ⑤	to 11.55 ⑥	to 12.04 ⑦	to 12.10 ⑧	to 12.20 ⑨	to 12.35 ⑩

Cardiopulmonary bypass commenced at 11.30 a.m., discontinued at 12.04 p.m.

TIME	OESOPHAGEAL TEMPERATURE °C	ARTERIAL BLOOD PRESSURE. mmHg
10.00		145
.15		140
.30	35.6	138
.43	34.6	136
11.30	32.8	
.34	31.9	
.35	31.7	95
.38		110
.42	31.1	110
.43	31.1	110
.44		110
.48	30.9	110
.51	30.9	105
.53	31.1	105
.55	31.1	100
.56		105
12.04		100
.05	30.9	104
.07	30.8	100
.11	30.6	100
.14	30.4	104

TIME	OESOPHAGEAL TEMPERATURE °C	ARTERIAL BLOOD PRESSURE mmHg
12.16	29.8	108
.18	29.8	108
.19	29.7	110
.21		100
.23		100
.25		104
.27		104
.28	31.0	
.31	30.8	
.35	30.9	105
.39	30.9	100

OBSERVED DATA.

URINE

URINE No.	1	2	3	4	5	6	7	8	9	10
Volume (ml.)	22.4	33.4	18.2	37.2	18.0	15.3	9.1	8.4	17.2	47.6
Osmolality (mOsm/L)	224	321	404	410	421	494	495	456	368	287
Creatinine (mg/100 ml)	106.6	105.0	128.3	103.3	100.0	74.1	82.5	83.3	60.3	46.2
Na (meq/L)	69.8	67.0	69.8	97.6	77.0	58.0	17.0	6.35	14.0	19.4
K (meq/L)	12.6	11.2	14.4	12.0	13.3	14.0	11.3	10.5	12.4	9.4
Ca (mg/100 ml)	6.0	-	6.1	7.4	6.1	5.9	4.3	3.8	2.8	1.8
Mg (mg/100 ml)	3.26	2.82	3.28	2.33	2.07	2.50	2.02	1.35	0.83	0.85
≠ Protein (mg/100 ml)	o	o	o	o	o	o	o	o	o	o
Inorganic Phosphate (mg/100 ml)	1.09	.98	.98	.80	.98	.83	.50	.35	.35	.24

≠ Protein is measured to the nearest 10 mg./100 ml.

BLOOD

Blood Sample	1	2	3	4	5	6	7
Osmolality (mOsm/L)	-	296	295	-	299	-	300
Creatinine (mg/100 ml)	6.81	6.72	6.38	4.22	4.57	5.09	5.26
Na (meq/L)	140.0	140.0	138.5	-	135.0	134.0	135.0
K (meq/l)	3,85	4.13	3.25	-	3.67	2.98	2.32
Ca (mg/100 ml)	10.9	-	10.9	-	10.1	-	10.3
Mg (mg/100 ml)	1.61	1.59	1.44	-	1.70	1.33	1.34
Inorganic Phosphate (mg/100 ml)	4.47	4.04	4.07	-	3.60	3.64	3.68

Plasma haemoglobin at conclusion of experiment: 40 mg/100 ml.

DERIVED DATA.

PERIOD	1	2	3	4	5	6	7	8	9	10
Urine volume (ml/min)	2.24	2.09	1.82	1.81	1.80	1.02	1.01	1.40	1.72	3.17
Midperiod Serum: Creatinine (mg/100 ml)	6.79	6.75	6.63	6.13	5.17	4.38	4.58	4.61	5.14	5.26
Na (meq/L)	140.0	140.0	139.6	138.2	136.9	135.8	134.8	134.2	134.3	135.0
K (meq/1)	3.90	4.04	3.91	3.29	3.45	3.57	3.54	3.14	2.79	2.32
Ca (mg/100 ml)	10.9	10.9	10.9	10.8	10.5	10.3	10.1	10.2	10.2	10.3
Mg (mg/100 ml)	1.61	1.60	1.54	1.56	1.64	1.63	1.63	1.42	1.33	1.34
Inorganic Phosphate (mg/100 ml)	4.39	4.17	4.05	3.86	3.72	3.62	3.62	3.63	3.65	3.68
Average Temperature (°C)	35.2	34.3	33.8	33.1	31.9	31.0	30.9	30.7	30.1	30.7
Ccr (ml/min)	35.1	32.5	35.2	30.6	34.9	17.3	18.2	25.3	20.2	27.8
% Excretion of Filtered										
Na	3.2	3.1	2.6	4.2	2.9	2.5	0.7	2.6	0.9	1.6
K	20.6	17.9	19.0	21.4	17.7	23.1	17.8	18.5	37.9	46.2
Ca	3.5	-	2.9	4.1	3.0	3.4	2.4	2.1	2.3	2.0
Mg	12.9	11.3	11.0	8.8	6.5	9.0	6.9	5.3	5.3	7.2
P	1.6	1.5	1.3	1.2	1.5	1.3	0.8	0.5	0.8	0.7
H_2O	6.4	6.4	5.2	5.9	5.2	5.9	5.6	5.5	8.5	11.4

CONCLUSIONS.

In devising these experiments, our purpose had been to delineate the effects of cardiopulmonary bypass on normothermic renal function (Dog 1) and on body temperature (Dog 2). We anticipated that the dogs would spontaneously maintain a more or less normal body temperature until the commencement of bypass. This was not the case. Dog 1 dropped its pre-bypass oesophageal temperature to 34°, and Dog 2, to 32.8°.

Enzyme activity usually falls off rapidly above and below a narrow range of optimum temperature (cf Appendix E) Assuming the optimum temperature for activity of renal enzyme systems to co-incide with the normal body temperature, it is plainly inadmissable to compare renal function at more than a few degrees below optimum, with normal values.

In other words, our experience with these 2 dogs made it clear that 'control' urines collected pre-bypass, were not controls at all, but were in fact marred by the effects of mild hypothermia of varying and unpredictable degree. It became necessary therefore to re-examine our objectives - what exactly were we trying to do?

My overall plan was straightforward; to measure a

few parameters of renal function at varying temperatures, and, of lesser importance, at comparable temperatures before and after body cooling. The surgeon's aim was even more modest; they wished largely to assure themselves of lack of ill effect of cardiopulmonary bypass and profound hypothermia, on renal function.

There seemed to be two alternatives. We could endeavour to improve the surgical technique, by calling for faster and more exacting surgery in a smaller thoracotomy wound, with the frequent application of hot packs. These measures might perhaps help to maintain the normal body temperature, pre- and post-bypass, but would be unlike the surgeons usual operative procedure. The interpretation of our results would then not be applicable to their normal work. Or we could accept the limitations of the existing technique (and so satisfy the surgeons) and I could confine my observations on renal function to the bypass period, during both cooling and rewarming. In so far as my interests lay, not in the effects of bypass, but rather in those of hypothermia, such a procedure would fulfil my purposes.

The surgeons professed themselves satisfied with comparison of pre- and post-bypass renal function, and were - from their point of view, rightly so - not concerned with the mild hypothermia induced pre-bypass.

In short, we abandoned our initial plan of clearly establishing the renal effects of normothermic bypass. This meant that should our subsequent experiments disclose renal malfunction after combined cardiopulmonary bypass and profound hypothermia, we might have difficulty in assessing which of these procedures was to blame.

CHAPTER VI.

DOG C.

Our experience with Dog B had satisfied us that we had an experimental design capable of elucidating those aspects of renal function which we wished to study. We now planned to repeat this procedure in a number of dogs, so as the better to evaluate the consistency and reproducibility of our results. Dog C was the first such dog.

EXPERIMENTAL PROCEDURE:

The procedure adopted here was identical to that followed in Dog B, with the following exceptions.

Urine was obtained via a short polyethylene catheter placed in the right ureter. This had been exposed through a small flank incision and dissected out retroperitoneally. The catheter was so placed that its tip lay well within the renal pelvis.

After an equilibration period of some 75 minutes, three control urine samples were collected, the first 40 minutes prior to commencement of cardiopulmonary bypass.

Two urine collections were made during the initial bypass period, before commencement of deliberate blood

stream cooling. The temperature was then lowered 'step-wise' i.e. the oesophageal temperature was held as close to 25° as possible, for 13 minutes, before dropping it to 10°.

On commencement of rewarming, during profound hypothermia, we attempted a 'stop-flow' experiment. The ureteric catheter was occluded for 2 minutes, and the urine ensueing immediately after release of the occlusion, collected. As had been the case in Dog B, the urine flow rate now seemed inadequate, and the attempt was abandoned. The next urine collection period was begun without delay.

Urine was collected up to 21 minutes after discontinuation of bypass.

RESULTS:

The results are shown graphically in Figs. 9, 10 and 11. Details of experimental and derived data are given at the end of this Chapter.

URINARY FLOW RATE AND OSMOLALITY, Cer, TmPAH, ARTERIAL BLOOD PRESSURE, AND OESOPHAGEAL TEMPERATURE. (FIG. 9)

During the control 40 minutes before bypass, urine flow rate ranged between 4.5 ml/min to 4.9 ml/min; and the urinary osmolality was at (314 mOsm/l) or slightly

below (280 mOsm/l., 299 mOsm/l) that of the serum. The Ccr showed a slight progressive fall, as did the TmPAH, but immediately pre-bypass these were still well within the normal range (cf Appendix C).

Periods 4and 5 embrace the first 10 minutes after commencement of bypass, before blood-stream cooling was begun. Urine flow rate, Ccr and TmPAH all fell sharply to less than half their pre-bypass values, while urinary osmolality rose from 299 mOsm/l to 380 mOsm/l. The oesophageal temperature fell from 34.0° to a plateau of about 28°. This was accompanied by a steep fall in arterial systolic blood pressure, from 130 mm Hg to 60 mm Hg.

Blood stream cooling was begun paripassu with urine collection period 6, and the temperature held, by alternate cooling and warming of the extracorporeal blood, to 25.9° - 24.7°, during periods 6, 7 and 8. Urine flow rate and osmolality returned to pre-bypass levels, while the Ccr showed little change. The TmPAH fell further, to 211 mg/min. during period 8.

During periods 9 to 12, the dog was cooled further, the oesophageal temperature reaching a low of 11.0° during period 12. Urine flow rate fell slightly, but remained

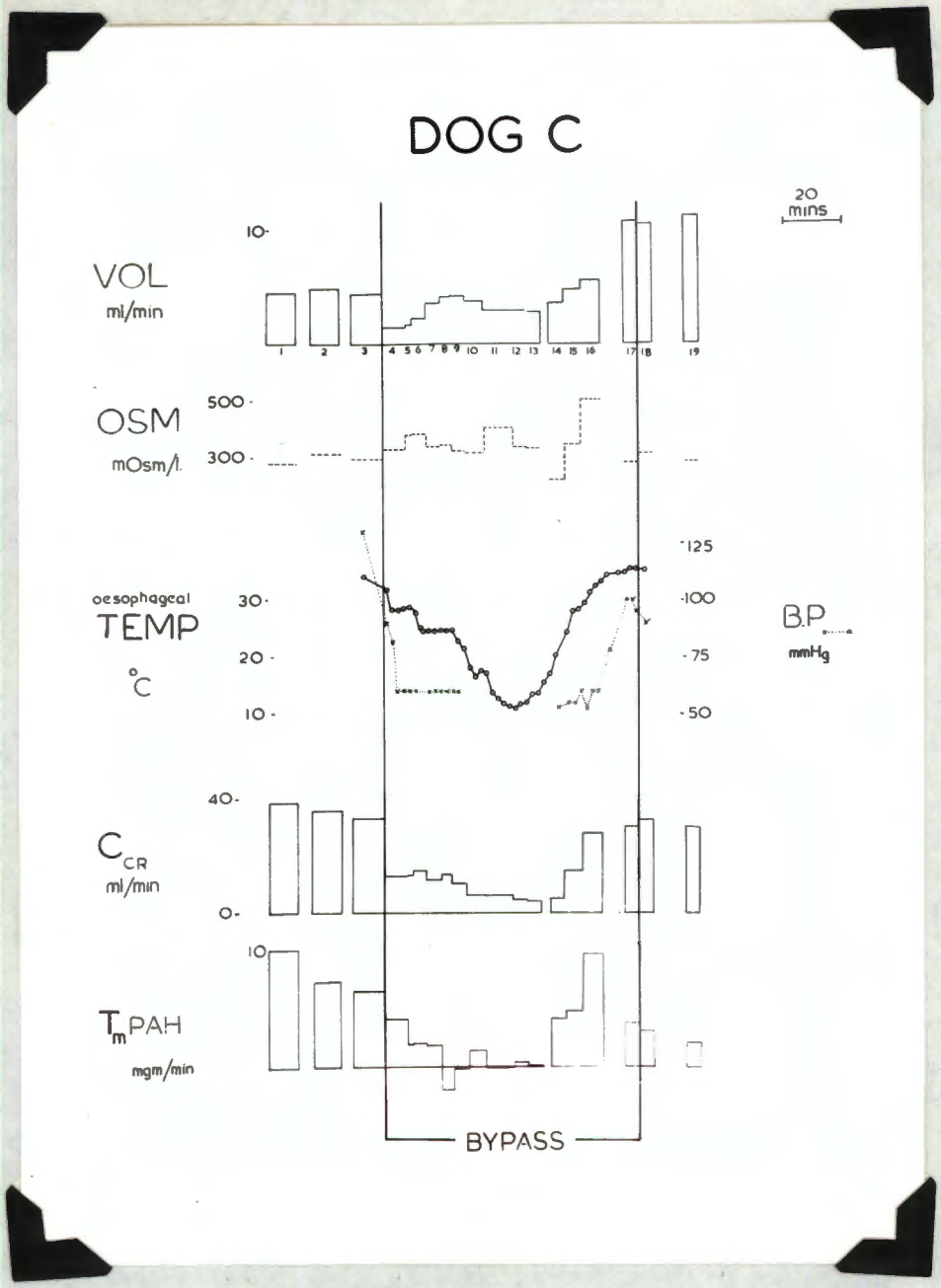

DOG C
20 mins
VOL
ml/min
10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
OSM
mOsm/l.
500
300
oesophageal
TEMP
°C
30
20
10
125
100
75
50
B.P
mmHg
C CR
ml/min
40
0
Tm PAH
mgm/min
10
BYPASS

FIG. 9.

above that of the initial bypass period. Urine osmolality rose abruptly to 403 mOsm/L during period 11, only to fall again to 339 mOsm/L in period 12. Serum osmolality at this time was 328 mOsm/L. The Ccr fell to about 4 ml/min. TmPAH oscillated irregularly just above and below zero. The C_{PAH} during periods 8 to 13, approximated to the Ccr.

Blood stream cooling was stopped after period 12, and during period 13 the oesophageal temperature rose, spontaneously, from 11.8° to 13.5°. The abortive attempt at a 'stop-flow' experiment was made between periods 13 and 14. The urine collected immediately after ureteric occulusion (period 14) showed a marked drop in urinary osmolality from 335 mOsm/l to 221 mOsm/litre. TmPAH rose abruptly from .01 mg/min. to 4.17 mg./min. As active blood stream rewarming proceeded-periods 14 to 16 - both the urine flow rate, and osmolality, increased, to levels higher than any previously attained in this experiment. This was accompanied by a progressive rise in Ccr and TmPAH.

The period immediately before termination of bypass - the mean oesophageal temperature was now 35.1°-was marked by a high urine flow rate (more than 10 ml/minute), low urinary osmolality, and a Ccr almost back to the pre-bypass

control level. The TmPAH however, fell. Arterial blood pressure rose in parallel with the temperature between periods 15 to 17.

The findings during post-bypass periods 18 and 19 were similar to those of period 17.

URINARY ELECTROLYTE AND PAH CONCENTRATION CHANGES. FIG 10.

Pre-bypass, the urine Na concentration varied between 28.4 meq./l and 39.8 meq./l. During the initial bypass periods 4 and 5, before active cooling was begun, this rose to 49.2 meq./l. Blood stream cooling brought it up to 71.4 meq/L (period 8 - mean oesophageal temperature 24.7°). Further reduction of oesophageal temperature to 14.5° (period 11) raised the urine Na concentration to 122.5 meq./L. During period 12 (mean oesophageal temperature 11.3°) it fell again to 100 meq./L. Rewarming (periods 13 to 17) saw a sharp drop in urine Na concentration, to as low as 5.8 meq/L. Thereafter it rose gradually, to reach 65.6 meq./L. during period 19.

Bypass produced little change in urine K concentration, from the pre-bypass levels of 6.6 - 7.8 meq./L. Cooling to 11.3° reduced this slightly, to 3.6 meq./L - 4.0 meq./l On rewarming, urinary K concentration rose once again, to 6.5 meq./L. Post-bypass urine collection periods had urine K concentrations of 10.6 meq/L and 3.1 meq/L.

Urinary Mg concentration hardly differed from control values, until blood stream cooling was begun; at 25°, it fell from about 1.5 mg/100 ml to 1.07 mg/100 ml. (period 8). Lowering the temperature further, raised the urinary Mg concentration to 1.83 mg./100 ml (period 11) and 1.48 mg/100 ml (period 12). Rewarming

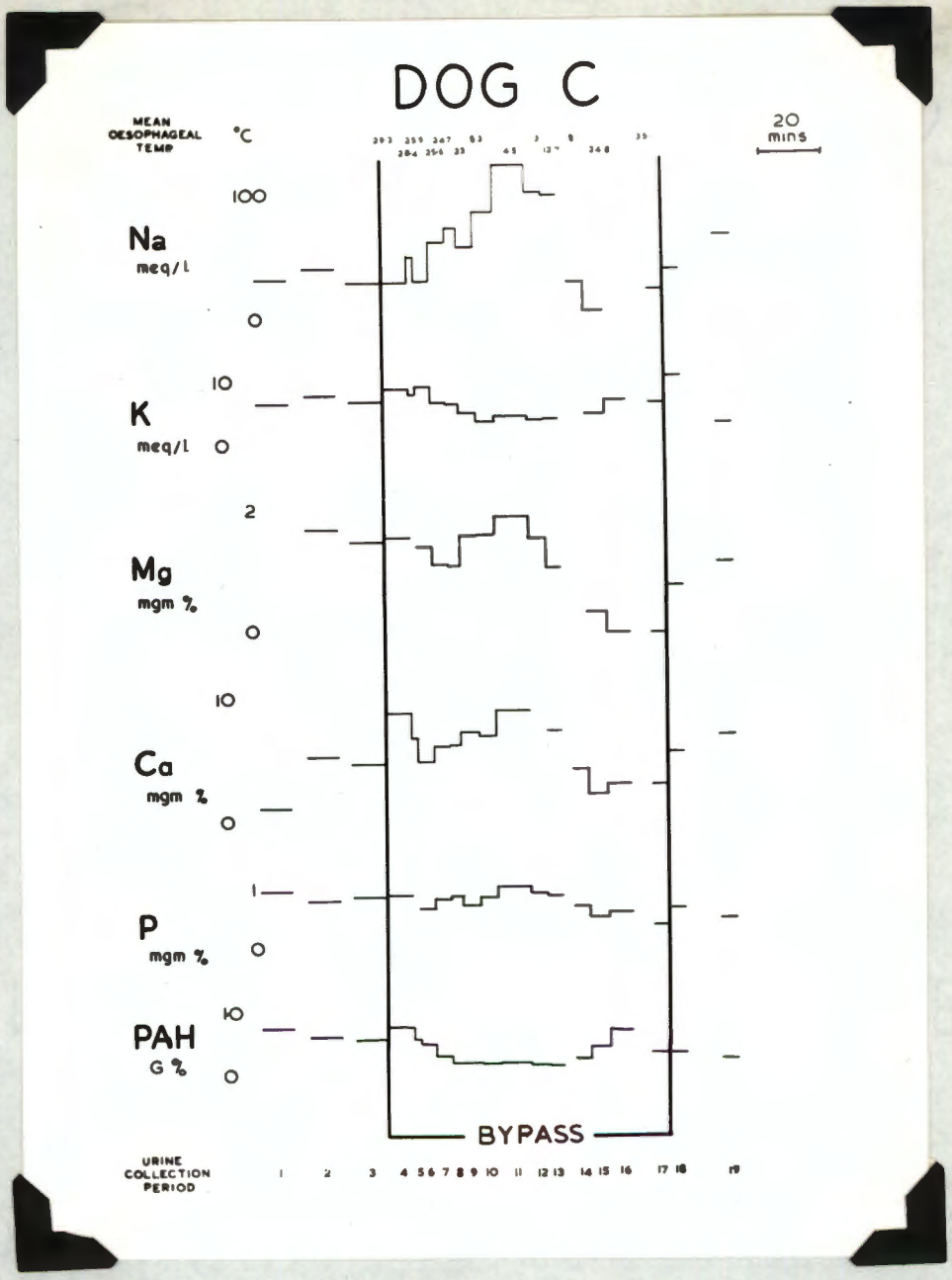

DOG C
MEAN OESOPHAGEAL TEMP
°C
20 mins
Na
meq/l
100
0
K
meq/l
10
0
Mg
mgm %
2
0
Ca
mgm %
10
0
P
mgm %
1
0
PAH
G %
10
0
BYPASS
URINE COLLECTION PERIOD
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

FIG. 10.

led to a big drop in urine Mg concentration, so that at 30.0° and 35.1° (periods 16 and 17) Mg was not detectable in the urine. Cessation of bypass brought about its reappearance, and urine Mg concentration rose to 1.1 mg./100 ml.

Control periods 1 to 3 saw a rise in urine Ca concentration, from 1.1 mg/100 ml, to 5.2 mg/100 ml and 4.7 mg/100 ml. This rise continued during the initial bypass period, then fell, only to rise again with cooling to 8.8 mg/100 ml at a mean oesophageal temperature of 14.5°. On rewarming, the urine Ca concentration fell sharply to 2.1 mg/100 ml and 2.9 mg/100 ml (periods 15 to 17). On cessation of bypass, this rose to 6.8 mg/100 ml.

Urine inorganic phosphate concentration showed little variation throughout the experiment. Rewarming, on bypass, was accompanied by a slight drop from 0.97 - 0.87 mg/100 ml (periods 11 and 12), to 0.54 - 0.34 mg/100 ml (periods 16 and 17),

The concentration of PAH fell during collection periods 1, 2 and 3. Bypass produced a transitory rise. Cooling was accompanied by a further fall, but never to below 130 mg/100 ml. (period 13). Rewarming raised this concentration, but by period 17 it fell again to 33 mg/100 ml. After resumption of the normal circulation it fell further, to 24 mg/100 ml.

PERCENTAGE EXCRETION OF FILTERED WATER AND ELECTROLYTES. FIG. 11.

The excretion of filtered Na varied between 2.8% and 3.9%, during periods 1 to 3, and remained at this level for the first 10 minutes after bypass was instituted. On initiation of blood-stream cooling, this rose to 15.8%, at a mean oesophageal temperature of 24.7° (period 8), and 54.5% at 11.3° (period 12). The rise continued during the succeeding period of slight spontaneous rewarming, (period 13 - mean oesophageal temperature 12.7°) to 58.4%. Active rewarming rapidly lowered this figure to 1.7%, at 24.8° (period 15). As rewarming progressed, the percentage excretion of filtered Na rose again, to finally reach 17.4% some sixteen minutes after discontinuation of bypass (period 19).

Water excretion followed a somewhat different course. Pre-bypass, 11.7% to 13.8% of filtered H_2O was excreted. It remained at about this level during periods 4 and 5, but rose to 30.2% at 25° (period 8). At 11.3° it reached 64.2%; and on rewarming first rose to 71.5% (period 14 - mean oesophageal temperature 18.1°) before falling to 19.8% at 30°. During the ensueing three normothermic periods, both on and off bypass, the excretion of filtered water varied between 32.4% and 36.8%. Inspection of Figure 6 reveals that the plot of percentage excretion

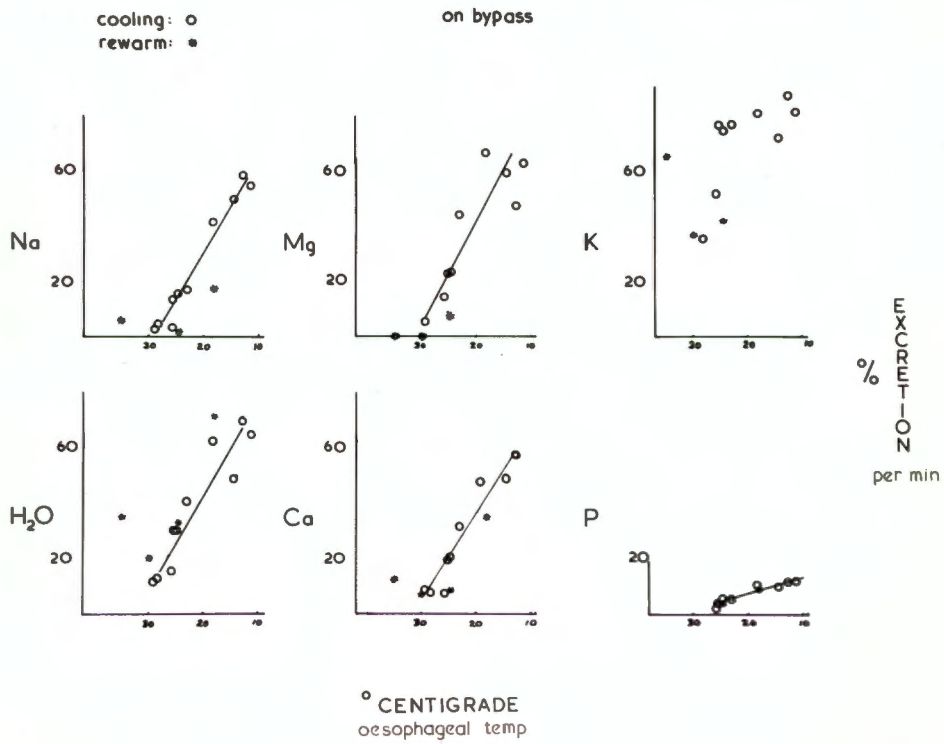

DOG C
on bypass
cooling: o
rewarm: *
Na
Mg
K
H_2O
Ca
P
60
20
% EXCRETION
per min
° CENTIGRADE
oesophageal temp

FIG. 11.

of filtered H_2O, against mean oesophageal temperature, shows some scatter, and that the percentage excretion, obtained on rewarming, for any given temperature, was greater then during cooling.

The percentage excretion of filtered Mg. fell from 16.1% and 13.1%, to 5.9% on bypass alone. Active blood stream cooling raised these figures to 14%, 22% and 23%, at 25°; and to 63% at a mean oesophageal temperature of 11.3° (Period 12). Unlike Na and H_2O, in this experiment, the % excretion dropped during period 13, and then fell to zero with normothermic perfusion. After re-establishment of the normal circulation, 16.1% and finally 25.2% of filtered Mg appeared in the urine. The plot of percentage excretion against temperature, while on bypass, shows some scatter, with no obvious difference between values derived during cooling, from those got during rewarming.

Calcium behaved rather differently. The initial bypass period produced perhaps a slight rise in percentage excretion. Active cooling to 25°, raised this further, from 7.9% to 18.6% and 20.0%. At 12.7° (period 13), 57% was excreted; this dropped to 7% on active rewarming. It finally rose, before the end of bypass, to reach 29.3%

during period 19. The plot of % excretion v. temperature while on bypass (Fig. 7) shows less scatter than was the case for Mg. There is no apparent difference between the percentage excretion on cooling and rewarming.

Bypass produced no change in percentage excretion of filtered K, until the temperature was brought down to 25°, when 76% and 74% were excreted. At 11.3°, this figure rose to 81%. Like Na and H_2O, this rose still further during period 13, (to 87%); unlike the case with these substances, however the percentage excretion of filtered K fell but tardily on active rewarming. By 30°, 37.1% was still excreted. Thereafter, the percentage excretion of K fluctuated irregularly, between 65% and 37.7%. Fig. 7 shows the correlation between percentage excretion of K, and temperature, to be less well defined than is the case for the other electrolytes and water in this experiment.

The percentage excretion of inorganic phosphate rose from 1.7, 3.9 and 4.7%, at about 25°, to 11.6% at 11.3°. The slight rise in temperature during period 13 produced no change in percentage excretion of inorganic phosphate. On active rewarming, this fell to 3.2% at 24.8°. The variation in percentage excretion of filtered inorganic

phosphate, with temperature, is much less than that of the other electrolytes, and water.

SERUM CONCENTRATION CHANGES:

Serum osmolality rose, from 313 mOsm/l. to 330 mOsm/l, just before bypass was discontinued.

Creatinine and PAH showed parallel concentration changes. Both dropped from steady control levels upon commencement of bypass. They then rose, progressively, during hypothermia and rewarming, until their concentration almost doubled. After bypass was discontinued, their serum concentrations fell.

The serum Na varied between 132.5 meq./l and 140 meq/l. before bypass. During hypothermia, it fell, irregularly, to 115.5 meq./l, but rose again to 129 meq/l on rewarming. This rise continued, to 139 meq./l. on resumption of normal circulation.

Serum K concentration fluctuated irregularly throughout the experiment, between 2.56 meq/l and 3.56 meq/l. Serum Mg also varied irregularly, but over a much narrower range (1.38 mg/100 ml to 1.61 mg/100 ml).

Both the serum Ca (11.7 mg/100 ml to 13.0 mg/100 ml) and inorganic phosphate (6.56 mg./100 ml) levels were

surprisingly high, before bypass. Both fell steadily throughout the bypass period. After discontinuation of bypass, serum Ca concentration rose, slightly, from 7.8 to 8.5 mg./100 ml.

COMMENT:

While the arterial blood pressure was monitored throughout the experiment, the actual readings were not recorded during urine collection periods 11 to 13, "as they remained constant". This failure to record the seemingly obvious occurred occasionally in other experiments too. I have only myself to blame for not impressing strongly enough upon the technician in charge, the necessity for continuous recording of such temperature and blood pressure data.

The urinary flow rate of almost 3 ml/minute during period 13, filled me with the hope that a stop-flow experiment would be successful, despite our experience to the contrary in Dog B. Upon release of the ureteric occlusion however, a gush of urine, usual on such occasions, did not occur. This presumably was due to the low arterial systolic blood pressure. Rightly or wrongly, this made me query the feasability of the procedure at the time, and I therefore abandoned the project as abruptly as I had embarked upon it.

Both serum creatinine and PAH concentrations dropped immediately bypass was instituted, as anticipated. They then rose, far more rapidly than expected, to almost double their initial concentrations. While there had been no obvious increase in actual drip rate, the rate of intravenous infusion was clearly too fast for the diminished urinary loss. Looking back at our results on the dogs previously experimented upon, we realised that 2 of the 3 dogs given similar doses of creatinine and PAH had also exhibited this progressive rise in serum creatinine and PAH concentrations during bypass. It has already been pointed out elsewhere, (Chapter 11) that any reduction in drip rate during bypass must be entirely arbitrary, as there are no immediate means of assessing the fall in GFR on bypass. I decided therefore, in future experiments, to stop the sustaining infusion of creatinine and PAH, entirely, at the beginning of the bypass period. The results could be of assistance in assessing just how much of these substances needed to be infused during bypass and profound hypothermia, so as to keep the serum concentrations fairly constant.

EXPERIMENTAL DATA ON DOG C.

WEIGHT: 16.9 kg.

INFUSIONS: 1. Dextrose Water - from 1.15 pm to 4.32 pm., at 9 ml./minute. Then replaced by N. saline, at same rate.

2. Creatinine 1.5 G)
PAH 2.4 G) in 900 ml. N. saline, at 3.8 ml/minute.

PRIMED at 1.30 p.m. with creatinine 0.5 G, PAH 1.8 G.

EQUILIBRATION PERIOD: 75 minutes

URINE COLLECTION: via short polyethylene catheter tied in right ureter, catheter tip within renal pelvis.

TIMES OF BLOOD AND URINE COLLECTIONS:

BLOOD SAMPLES:	(1) 2.51	(2) 3.06	(3) 3.17	(4) 3.31	(5) 3.39	(6) 3.48	(7) 4.03
Urine collection periods;	2.46 pm to 2.56 pm (1)	3.01 to 3.11 (2)	3.15 to 3.26 (3)	to 3.34 (4)	to 3.36 (5)	to 3.41 (6)	to 3.46 (7)	to 3.49½ (8)	to 3.54½ (9)	to 4.01 (10)	to 4.11 (11)

BLOOD SAMPLES:	(8) 4.15			(9) 4.31			(10) 4.53			(11) 5.13	
Urine collection periods:	to 4.16 (12)	to 4.21 (13)		4.24 to 4.29 (14)	to 4.35 (15)	4.42 (16)		4.50 to 4.55 (17)	to 5.00 (18)		5.11 to 5.16 (19)

Cardiopulmonary bypass was begun at 3.26 pm., bloodstream cooling at 3.36 pm., rewarming at 4.24 p.m., and bypass was discontinued at 4.55 pm., with resumption of normal circulation.

A stop-flow experiment was attempted between periods 13 and 14. Urine flow rate after release of the occluded ureteric catheter was too slow however and the attempt was abandoned.

OBSERVED DATA:

TIME	OESOPHAGEAL TEMPERATURE °C	ARTERIAL BLOOD PRESSURE mm HG.
3.19	34.0	130
3.27	31.8	90
.29	28.1	82
.31	28.2	60
.33	28.5	60
.35	28.7	60
.37	27.7	60
.39	25.0	-
.40	24.5	-
.42	24.6	60
.44	24.6	60
.46	24.7	60
.48	24.7	60
.50	24.8	60
.52	22.7	-
.54	21.4	-
.58	18.0	-
4.00	16.4	-

TIME	OESOPHAGEAL TEMPERATURE °C	ARTERIAL BLOOD PRESSURE mm Hg.
4.02	17.7	
.04	17.0	
.06	13.6	
.08	12.6	
.10	11.8	
.12	11.2	
.14	11.0	
.16	11.8	
.18	12.0	
.20	13.3	
.22	13.6	
24	15.5	
26	17.0	
28	20.2	53
32	24.3	55
34	28.0	55
36	28.3	60
38	29.4	52.5

OBSERVED DATA: (contd.)

TIME	OESOPHAGEAL TEMPERATURE °C	ARTERIAL BLOOD PRESSURE mm Hg.
.40	31.2	60
.42	32.2	60
.44	33.2	-
.46	34.4	78
.50	34.7	-
.52	34.9	100
.54	35.6	100
.55	35.5	95
4.58	35.1	90

4.48: 50 ml of $NaHCO_3$ given I.V.

URINE

Urine No.	1	2	3	4	5	6	7
Volume (ml)	45.0	49.0	49.3	11.7	3.3	11.3	17.7
Osmolality (mOsm/l)	280	314	299	328	380	382	340
Creatinine (mg/100 ml)	40.0	34.0	34.0	37.0	37.0	32.5	18.0
Na (meq/L)	31.2	39.8	28.4	28.0	49.2	29.5	60.5
K (meq/L)	6.6	7.8	6.8	8.7	7.8	9.1	6.7
Ca (mg/ 100 ml)	1.10	5.2	4.7	8.6	6.7	4.8	6.0
Mg (mg/ 100 ml)	-	1.66	1.46	1.51	-	1.37	1.08
* PAH (mg/ 100 ml)	2.93	2.34	2.24	2.93	2.18	1.83	1.06
‡ Protein (mg/100 ml)	o	o	o	o	o	o	o
Inorganic Phosphate (mg/100 ml)	0.92	0.74	0.80	0.81	-	0.61	0.77

URINE No.	8	9	10	11	12	13	14
Volume (ml)	14.5	21.2	25.0	29.8	14.9	14.3	18.3
Osmolality (mOsm/l)	344	325	319	403	339	335	221
Creatinine (mg/100 ml)	19.0	15.0	10.5	14.5	11.5	11.0	11.5
Na (meq/L)	71.4	56.5	85.0	122.5	100.0	98.0	28.5
K (meq/L)	6.3	5.0	3.6	4.3	3.8	4.0	-
Ca (mg/ 100 ml)	6.1	7.1	6.8	8.8	-	7.2	4.2
Mg (mg/ 100 ml)	1.07	1.54	1.56	1.83	1.48	1.02	-
* PAH (mg/ 100 ml)	0.71	0.70	0.64	0.67	0.59	0.53	1.01
≠ Protein (mg/100 ml)	o	o	o	o	o	o	o
Inorganic Phosphate (mg/100 ml)	0.81	0.65	0.80	0.97	0.87	0.81	0.66

Urine No.	15	16	17	18	19
Volume (ml)	29.3	38.8	53.9	52.5	56.0
Osmolality (mOsm/l)	346	505	283	317	289
Creatinine (mg/100 ml)	25.5	43.0	24.0	25.0	20.5
Na (meq/L)	5.8	-	22.0	38.0	65.6
K (meq/L)	4.7	6.9	6.5	10.6	3.1
Ca (mg/100 ml)	2.1	2.9	2.7	5.5	6.8
Mg (mg/100 ml)	0.32	0.0	0.0	0.75	1.10
* PAH (mg/100 ml)	1.63	2.80	1.30	1.30	0.97
‡ Protein (mg/100 ml)	o	o	o	o	o
Inorganic Phosphate (mg/100 ml)	0.48	0.54	0.34	0.61	0.46

* Urine was diluted X250 for PAH estimations; the values given are not corrected for this dilution.

‡ Protein is measured to the nearest 10 mg/100 ml.

BLOOD

BLOOD SAMPLES:	1	2	3	4	5	6	7	8	9	10	11
Osmolality (mOsm/L)	313	-	-	-	-	-	313	328	326	330	-
Creatinine (mg/100 ml)	4.68	-	4.78	4.20	5.11	5.75	6.92	7.45	8.50	8.55	7.55
Na (meq/L)	132.5	140.0	135.0	131.0	128.0	136.5	121.0	117.5	115.5	129.0	139.0
K (meq/L)	2.94	3.13	2.86	2.75	2.75	2.56	2.86	3.03	3.75	3.56	3.03
Ca (mg/ 100 ml)	11.9	11.7	13.0	-	-	9.2	8.8	8.9	8.4	7.8	8.5
Mg (mg/100 ml)	1.49	1.43	-	1.59	1.50	1.38	1.50	1.50	1.50	1.42	1.61
PAH (mg/ 100 ml)	54.0	65.0	63.5	54.0	62.3	75.5	-	96.5	111.4	114.3	91.0
Inorganic Phosphate (mg/100 ml)	6.56	-	-	-	5.68	5.20	4.80	4.81	5.04	3.84	-

Plasma Haemoglobin at end of experiment : 48 mg/100 ml.

DERIVED DATA.

PERIOD	1	2	3	4	5	6	7
Volume (ml/min)	4.50	4.90	4.48	1.46	1.65	2.26	3.54
Midperiod Serum:							
Creatinine (mg/100 ml)	4.68	4.74	4.63	4.24	4.66	5.05	5.43
Na (meq/L)	132.5	140.0	134.0	132.0	129.5	128.2	132.3
K (meq/L)	2.94	3.13	2.85	2.75	2.75	2.75	2.65
Ca (mg/100 ml)	11.9	11.7	12.6	11.4	10.8	10.3	9.7
Mg (mg/100 ml)	1.49	1.43	1.52	1.58	1.55	1.51	1.44
Inorganic Phosphate (mg/100 ml)	6.56	-	-	-	-	5.68	5.44
PAH (mg/100 ml)	64.0	65.0	61.3	55.0	58.0	61.7	69.0
Average Temperature (°C)	-	-	-	29.3	28.4	25.9	25.6
Ccr (ml/min)	38.46	35.45	32.90	12.74	13.10	14.54	11.73
C_{PAH} (ml/min)	51.4	44.2	40.8	19.5	16.8	16.7	13.6
TmPAH (mg/min)	10.4	7.5	6.6	4.2	2.0	2.1	1.9
% Excretion of Filtered:							
Na	2.8	3.9	2.9	2.4	4.8	3.6	13.8
K	26.3	34.4	32.5	36.3	35.7	51.4	76.3
Ca	10.8	6.2	5.1	8.7	7.9	7.3	18.6
Mg	-	16.1	13.1	5.9	-	14.1	22.6
P	1.6	-	-	-	-	1.7	3.9
H_2O	11.7	13.8	13.6	11.5	12.6	15.5	30.2

PERIOD	8	9	10	11	12	13	14
Volume (ml/min)	4.14	4.24	3.85	2.98	2.98	2.86	3.66
Midperiod Serum:							
Creatinine (mg/100 ml)	5.73	6.06	6.51	7.05	7.38	7.68	8.20
Na (meq/L)	136.3	132.4	126.4	120.1	117.9	117.1	116.1
K (meq/L)	2.56	2.64	2.76	2.90	3.01	3.19	3.55
Ca (mg/100 ml)	9.2	9.1	8.9	8.8	8.9	8.8	8.5
Mg (mg/100 ml)	1.38	1.41	1.46	1.50	1.50	1.50	1.50
Inorganic Phosphate (mg/100 ml)	5.21	5.09	4.94	4.80	4.81	4.86	4.97
PAH (mg/100 ml)	75.0	78.5	83.2	89.7	95.2	99.8	107.5
Average Temperature ^{o}C	24.7	23.0	18.3	14.5	11.3	12.7	18.1
Ccr (ml/Min)	13.73	10.50	6.21	6.13	4.64	4.10	5.13
C_{PAH} (ml/min)	9.85	9.37	7.40	5.60	4.58	3.78	8.57
TmPAH (mg/min)	2.14	-.16	1.40	-.07	.32	.01	4.17
% Excretion of filtered:							
Na	15.8	17.2	41.7	49.60	54.5	58.4	17.5
K	74.2	76.5	80.9	72.1	81.0	87.0	87.5
Ca	20.0	31.6	47.4	48.6	-	57.0	35.4
Mg	23.2	44.1	66.4	59.2	63.4	47.4	-
P	4.7	5.2	10.0	9.8	11.6	11.6	9.5
H_2O	30.2	40.4	62.0	48.61	64.2	69.8	71.5

PERIOD	15	16	17	18	19
Volume (ml/Min)	4.88	5.54	10.78	10.50	11.20
Midperiod Serum:					
Creatinine (mg/100 ml)	8.50	8.52	8.55	8.10	7.55
Na (meq/L)	116.1	120.1	128.7	133.5	139.0
K (meq/L)	3.74	3.69	3.56	3.35	3.03
Ca (mg/100 ml)	8.4	8.2	7.8	8.1	8.5
Mg (mg/100 ml)	1.50	1.47	1.42	1.51	1.61
Inorganic Phosphate (mg/100 ml)	4.99	-	-	-	-
PAH (mg/100 ml)	111.4	112.5	114.0	103.8	91.0
Average Temperature (^{o}C)	24.8	30.0	35.1	-	-
Ccr (ml/min)	14.64	27.96	30.26	32.41	30.41
C_{PAH} (ml/min)	17.80	34.45	30.61	32.84	29.83
TmPAH (mg/min)	4.90	9.9	3.9	3.2	2.2
% Excretion of filtered:					
Na	1.7	-	6.1	9.2	17.4
K	41.9	37.1	65.0	96.5	37.7
Ca	8.3	7.0	12.3	22.1	29.3
Mg	7.1	o	o	16.1	25.2
P	3.21	-	-	-	-
H_2O	33.3	19.8	35.6	32.4	36.8

CHAPTER VII.

DOG D.

Dog C had taught us that we were still infusing too much creatinine and PAH during the bypass period. In this dog, therefore, we resolved to stop the intravenous infusion immediately bypass was begun, and to resume it only when normal circulation was restored. We hoped that this would help to maintain fairly constant plasma levels of these substances.

In all other respects, the experiment on this Dog was similar to that on Dog C.

RESULTS:

The results are given graphically in Figs. 12, 13 and 14 and details of experimental and derived data are given at the end of this Chapter.

URINE FLOW RATE, OSMOLALITY, BLOOD PRESSURE, Ccr AND TmPAH (FIG. 12.)

Prebypass, the urinary flow rate was 0.99 ml/min., osmolality 392 mOsm/L., Ccr 12.4 ml/min, and TmPAH 21.5 mg/min. The B.P. was not recorded; the oesophageal temperature at the end of this period was 29.4°C.

Immediately bypass was commenced, the urinary flow

rate, C_{cr} and TmPAH dropped very markedly, to small fractions of their prebypass values. Urine osmolality, and oesophageal temperature, remained unchanged. It is of interest to note that the fall in Ccr, TmPAH and urine flow rate occurred despite a transient rise in BP., from 90 to 100 mmHg.

Period 3 marks the commencement of blood stream cooling. There was a rapid fall in oesophageal temperature, from 29° to a low of 10.2°, accompanied initially by substantial increases in urine flow rate, Ccr, and TmPAH. These then fell with the falling temperature, so that during period 6 they were at levels comparable to those prevailing just after bypass was begun (period 2). Osmolality and arterial B.P. fell sharply with cooling, to 336 mOsm/L and 45 mmHg respectively.

Rewarming reversed all the above changes, so that during period 9, just before recommencement of normal circulation, the urine flow rate, and Ccr were slightly higher than during period 1 (prebypass). Urinary osmolality rose to 436 mOsm/L. TmPAH rose too, but to a lesser extent than the Ccr.

After restitution of the normal circulation, urine flow rate increased while urinary osmolality dropped sharply. The Ccr and TmPAH fell slightly.

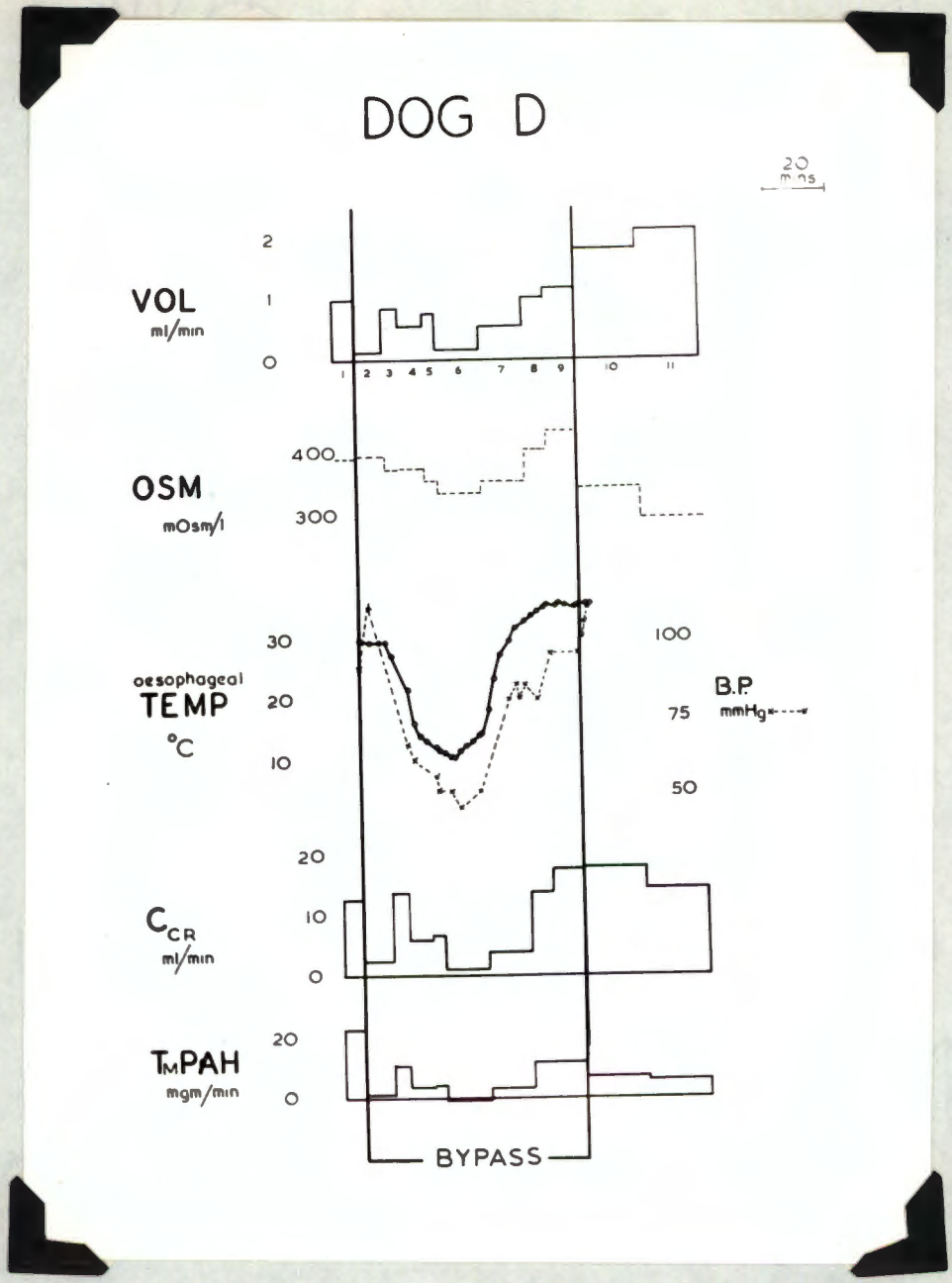

DOG D
20 mins
VOL
ml/min
2
1
0
1 2 3 4 5 6 7 8 9 10 11
OSM
mOsm/l
400
300
oesophageal
TEMP
°C
30
20
10
B.P
mmHg
100
75
50
C_{CR}
ml/min
20
10
0
T_MPAH
mgm/min
20
0
BYPASS

FIG. 12.

URINARY ELECTROLYTE AND PAH CONCENTRATION CHANGES. (Fig. 13)

Immediately bypass was begun (period 2), with no fall in oesophageal temperature, the urinary Na rose from 8.6 to 32.8 meq/l., while K fell from 8.0 to 1.6 meq/l., and PAH from 2.873 to 1.913 G/100 ml. The inorganic phosphate concentration was unchanged. Mg and Ca concentrations were not measured during period 2.

The commencement of blood stream cooling (period 3) saw a drop in Na concentration to 22.1 meq/l., while K rose abruptly to 11.0 meq/l. Inorganic phosphate remained unchanged, while PAH fell slightly, to 1.740 G/100 ml. The urinary Mg and Ca concentrations were 0.53 and 4.0 mg/100 ml., respectively.

Further cooling, to a mean oesophageal temperature of 12.2° (period 6) lead to a rise in urinary Na, to 102 meq/l., while Mg and Ca rose to 1.87 and 7.6 mg/100 ml respectively. K fell to 4.9 meq/l., and PAH to 0.640 G/100 ml. Inorganic phosphate fell from 1.64 to 0.90 mg/100 ml.

Rewarming (periods 7 to 9) reversed these changes, so that by period 9, Na, K and PAH concentrations were at levels approximating to those of period 3. Urinary Ca concentration fell to 5.2 mg/100 ml. Mg fell to 0.57

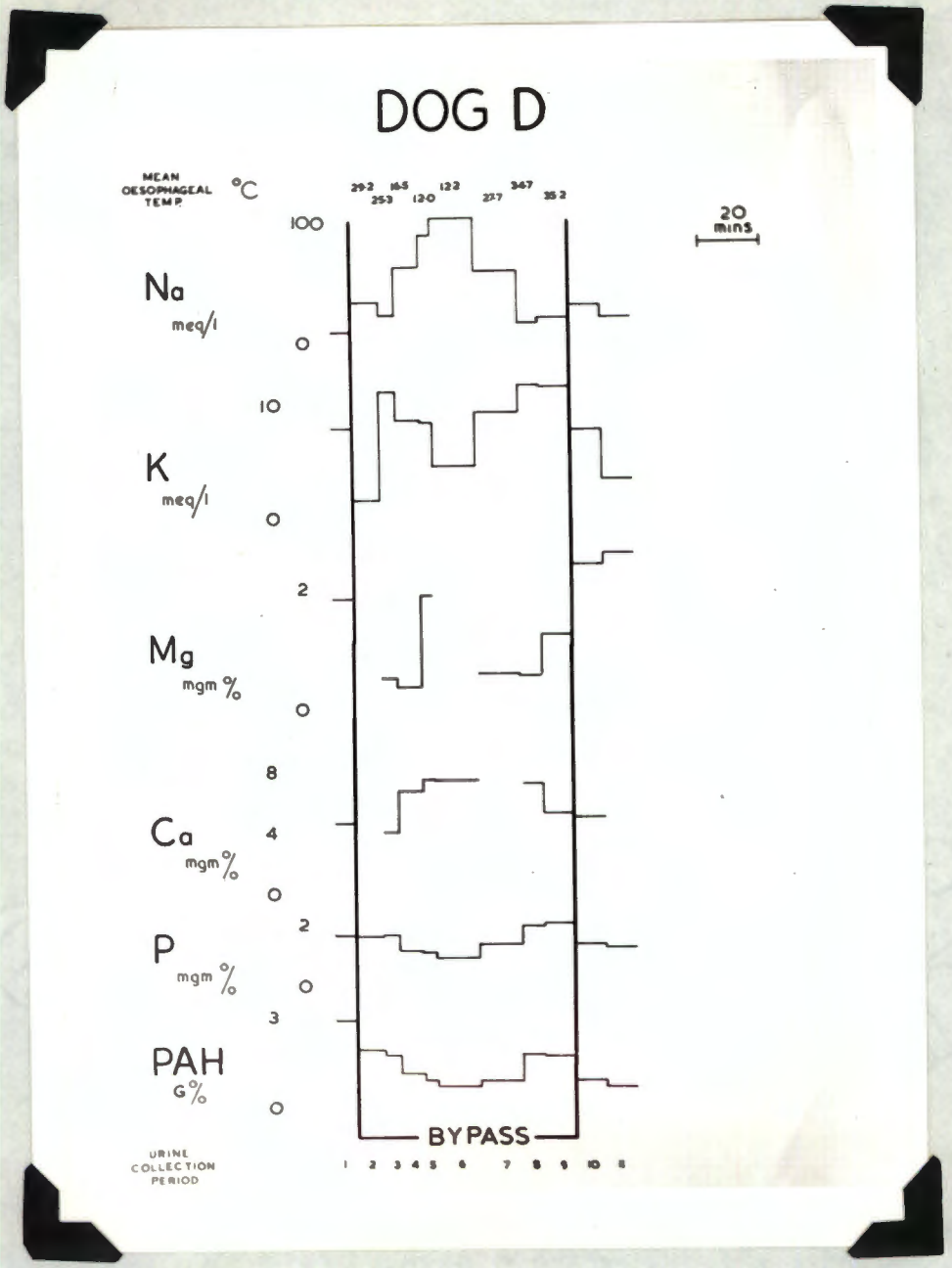

DOG D
MEAN OESOPHAGEAL TEMP °C
29·2 16·5 12·2 34·7
25·3 12·0 23·7 35·2
20 mins
Na meq/l
100
0
K meq/l
10
0
Mg mgm %
2
0
Ca mgm %
8
4
0
P mgm %
2
0
PAH G %
3
0
BYPASS
URINE COLLECTION PERIOD
1 2 3 4 5 6 7 8 9 10 11

FIG. 13.

mg/100 ml in period 8 (mean oesophageal temperature 34.7°) but rose thereafter. Inorganic phosphate rose to 2.03 mg/100 ml during period 9.

Post-bypass, K fell to 3.8 meq/l., Mg rose to 2.55 mg/100 ml, PAH fell to 0.685 G/100 ml., and inorganic phosphate fell to 1.24 mg/100 ml. Ca concentration was 4.9 mg/100 ml. /in period 10. Urinary Na was 20.0 meq/l at the conclusion of the experiment (period 11).

PERCENTAGE EXCRETION OF FILTERED WATER AND ELECTROLYTES (FIG. 14)

The percentage excretion of Na, H_2O, Ca and Mg rose with cooling. If one discriminates between the data obtained on cooling from those on rewarming, then the regression lines for Na, H2O and Ca percentage excretion are seen to be roughly linear. The curves drawn on the rewarming data lie above those for cooling, by some 5-10% excretion in all three instances.

The data for Mg are less satisfactory. There is a suggestion that they follow the course described for Na, H_2O and Ca, but other descriptions are equally possible.

Inorganic phosphate shows no variation in percentage excretion with falling temperature. A line drawn on the data obtained on rewarming is 1-2 per cent excretion higher than that drawn on the data obtained on cooling.

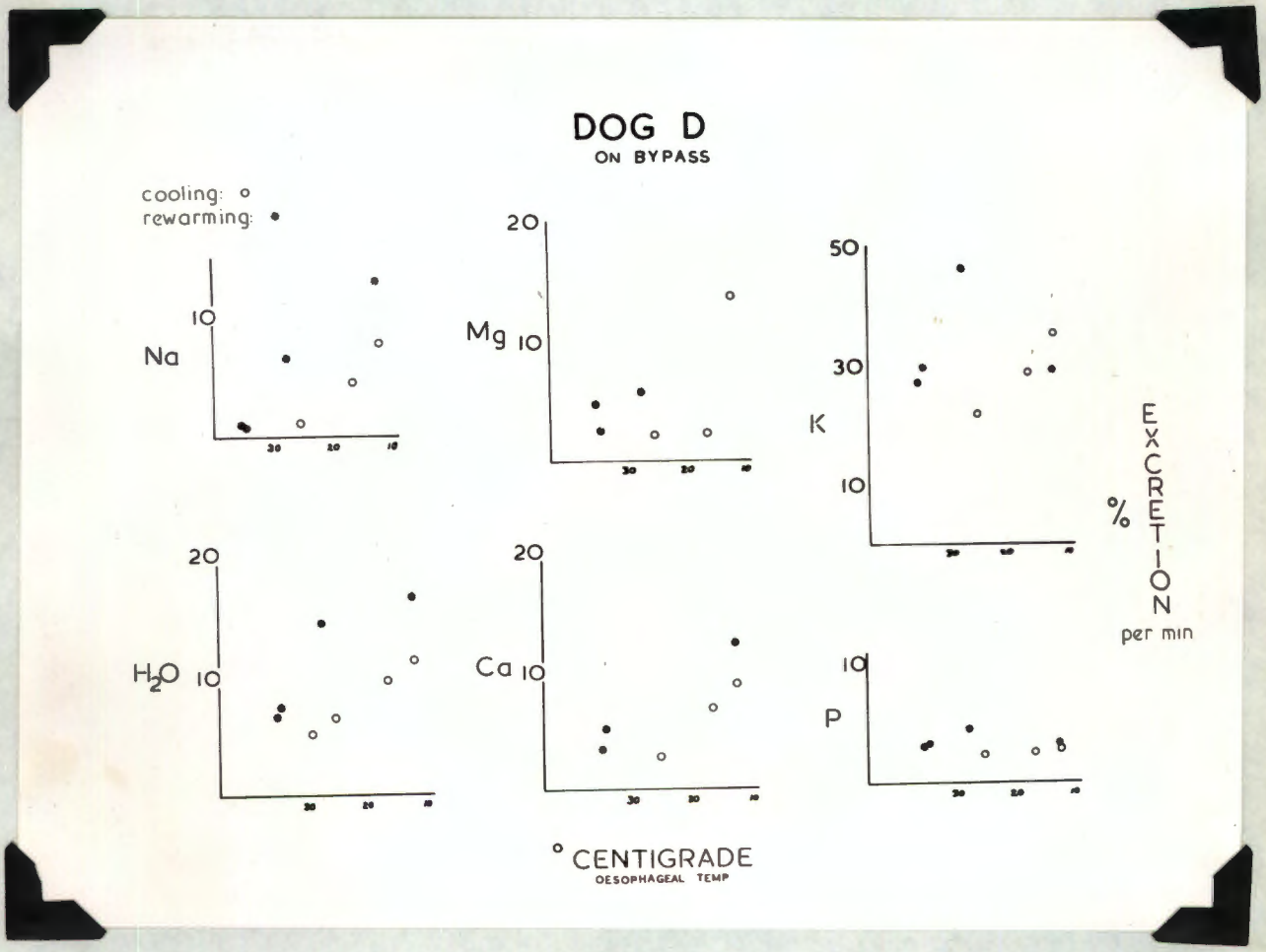

DOG D
ON BYPASS
cooling: o
rewarming: •
Na
Mg
K
H_2O
Ca
P
% EXCRETION per min
° CENTIGRADE
OESOPHAGEAL TEMP

FIG. 14.

There appears to be no correlation between temperature and percentage excretion per minute, of "filtered" K.

PROTEINURIA:

There was no protein in the urine, at the beginning of the experiment. A trace appeared on the initiation of blood-stream cooling (periods 3 and 4) but then disappeared on further cooling.

On active rewarming (period 7), proteinuria reappeared, (30-40 mg/100 ml), and rose to 80-90 mg/100 ml during period 8. In period 9, it fell to 40-50 mg/100 ml, and by the conclusion of the experiment was 30-40 mg/100 ml.

SERUM CONCENTRATION CHANGES:

Osmolality remained constant throughout, at 293-289 mOsm/Litre.

Serum creatinine dropped from 8.4 mg/100 ml to 5.3 mg/100 ml on institution of bypass, and then rose, with minor fluctuations, to 7.32 mg/100 ml. during the bypass period. Immediately post-bypass it was 7.0 mg/100 ml. and then rose to 7.5 mg/100 ml by the end of the experiment.

PAH followed a very similar course to that of creatinine, dropping from 60.6 mg/100 ml to 33.7 mg/100 ml

on beginning bypass, and then rising rapidly to 50.2 mg/100 ml. The final value, after resumption of normal circulation, was 71.0 mg/100 ml.

Serum Na concentration fell from 135 to 124.5 meq/l. during hypothermia, then rose to 132.5 meq/l. just before termination of bypass. Postbypass levels were 128.5 meq/l and 131.5 meq/l.

Serum K rose from 3.13 to 3.55 meq/l. on beginning bypass, then fell to 2.63 meq/l (blood 4). It then rose abruptly to 2.91 in blood 5, only to fall progressively to a final value of 2.56 meq/l.

Only 3 sera were tested for Ca concentration. This rose from 9.0 mg/100 ml, to 10.4 mg/100 ml. during hypothermia.

Serum Mg fluctuated irregularly between 1.71 mg/100 ml and 1.42 mg/100 ml. throughout the experiment.

The inorganic phosphate concentration of the serum rose from 4.00 mg/100 ml to 4.42 mg/100 ml on initiation of bypass, but thereafter fluctuated between 4.42 mg/100 ml and 4.32 mg/100 ml.

COMMENT:

The surgeons had previously noted, in passing, that

all the dogs into which I had infused PAH bled more freely than usual. In this dog, no sooner had the priming dose of PAH been given, than the surgeons complained of "almost uncontrollable capillary bleeding". This delayed their operative progress considerably - hence the equilibration period of 85 minutes. PAH is well known to cause autonomic reactions, when given intravenously in large doses. We resolved, therefore, that in future PAH would be given only after the major surgery had been completed.

The urinary flow rate was rather slow during profound hypothermia (0.18 ml/min. during period 6). Thinking the ureteric catheter might be obstructed, we moved it about a little; this lead to the appearance of fresh blood in the urine, fortunately in small amounts. Whether this readiness to bleed was indirectly brought about by the PAH is uncertain.

From the viewpoint of improving our technique, we were most interested to discover what would happen to the serum creatinine and PAH concentrations, on discontinuing the sustaining infusion at the time of commencement of bypass. The results (cf above) surprised us. The continued, yet irregular, rise during the bypass period, in the complete absence of administration

of additional creatinine or PAH, can only denote delayed and incomplete mixing of the unprimed blood in the extracorporeal circuit with that within the dog. On reflection, we should perhaps not have been so surprised; just as an equilibration period is needed with the usual techniques, so would it be needed here. The rise in serum concentration of creatinine and PAH, postbypass, is readily explained by the resumption of the 'sustaining' infusion.

EXPERIMENTAL DATA ON DOG D.

WEIGHT: 20.5 kg.

INFUSIONS: 1. 10% Dextrose water; at about 3.5 ml/min.
2. Creatinine 1.5 G)
PAH 2.4 G) in a litre of N saline. Run in at about 3.5 ml/minute.

PRIMED at 11.30 a.m., with creatinine 0.5 G., PAH 1.8 G.

EQUILIBRATION PERIOD: 85 minutes

URINE COLLECTION: via short polyethylene catheter tied into right ureter; catheter tip within renal pelvis.

TIMES OF BLOOD AND URINE COLLECTIONS:

BLOODS:	(1) 1.00	(2) 1.07		(3) 1.22		(4) 1.36	(5) 1.45	(6) 1.59		(7) 2.18	(8) 2.28
Urine collection Periods:	12.55 to 1.02 (1)	to 1.11 (2)	to 1.16 (3)	to 1.24 (4)	to 1.28 (5)	to 1.42 (6)	to 1.56 (7)	to 2.03 (8)	to 2.13 (9)	to 2.23 (10)	to 2.33 (11)

Cardiopulmonary bypass was begun at 1.02; blood stream cooling at 1.11; rewarming at 1.42; and bypass was discontinued at 2.13 p.m.

NOTES: (1) Blood appeared in the urine from urine specimen 7 onwards; this followed manipulation of the ureteric catheter in an attempt to improve the urine flow rate.

(2) The surgeons commented upon "a great deal of almost uncontrollable capillary bleeding" which appeared soon after the priming dose of creatinine and PAH had been given.

(3) Both infusions were stopped during the hypothermic period; that containing the creatinine and PAH at the moment of commencement of bypass, and that of 10% dextrose water four minutes later. Both infusions were recommenced when the bypass was discontinued, at 2.13 p.m.

OBSERVED DATA.

URINE

Urine No.	1	2	3	4	5	6	7	8	9	10	11
Volume (ml.)	6.9	1.1	4.3	4.3	3.1	2.5	7.8	7.1	11.7	18.0	21.0
Osmolality (mOsm/l)	392	396	375	376	357	336	356	405	436	346	295
Creatinine (mg/100 ml)	105	107	91.6	67.3	59.6	43.7	48.3	100	107	68.6	50.8
Na (meq/l)	8.6	32.8	22.1	61.8	88.0	102.0	58.5	15.7	21.0	30.5	20.0
K (meq/l)	8.0	1.6	11.0	8.7	8.5	4.9	9.3	11.5	11.3	7.8	3.8
Ca (mg/100 ml)	4.72	-	4.02	6.86	7.56	7.56	-	7.20	5.20	4.92	-
Mg (mg/100 ml)	1.81	-	0.53	0.38	1.87	-	0.60	0.57	1.24	2.38	2.55
≠ PAH (mg/100 ml)	11.49	7.65	6.96	4.47	3.65	2.76	3.48	6.96	6.84	3.59	2.74
Inorganic Phosphate (mg/100 ml)	1.64	1.62	1.64	1.15	1.11	0.90	1.35	1.96	2.03	1.35	1.24
* Protein (mg/100 ml)	o	o	tr	tr	o	o	30-40	80-90	40-50	40-50	30-40

≠ Urine was diluted X250 for PAH estimations; the values given are not corrected for this dilution.

* Protein is measured to the nearest 10 mg/100 ml.

BLOOD

Blood Sample:	1	2	3	4	5	6	7	8
Osmolality (mOsm/l)	293	287	289	287	-	-	-	289
Creatinine (mg/100 ml)	8.40	5.30	6.55	7.14	6.79	7.32	6.97	7.50
Na (meq/l)	-	135.0	129.5	125.0	124.5	132.5	128.5	131.5
K (meq/l)	3.13	3.55	2.78	2.63	2.91	2.83	2.75	2.56
Ca (mg/100 ml)	9.0	-	9.7	-	10.4	-	-	-
Mg (mg/100 ml)	1.46	1.60	1.54	1.58	1.46	1.71	1.63	1.42
PAH (mg/100 ml)	60.6	33.7	53.3	48.5	-	50.2	-	71.0
Inorganic Phosphate (mg/100 ml)	4.00	4.42	4.42	-	4.32	4.45	4.35	-

Plasma haemoglobin at conclusion of experiment: 112 mg/100 ml.

OBSERVED DATA.

Time	Oesophageal Temperature °C	Arterial Blood Pressure mmHg.
1.02	29.4	90
.05	29.2	110
.08	29.3	
.10	29.1	
.12	27.0	
.17	21.5	65
.19	16.0	60
.21	14.0	
.23	13.2	
.26	12.1	55
.27	11.7	50
.29	11.3	
.31	10.6	50
.32	10.2	
.34	11.7	45
.36	12.5	
.38	13.1	
.40	14.1	50
.41	14.5	
.43	18.2	
.45	23.2	
.47	27.2	
.50	29.5	80

Time	Oesophageal Temperature °C	Arterial Blood Pressure mmHg
1.52	31.6	85
.53	32.8	80
.55	33.6	85
.57	34.1	
.59	34.9	80
2.00	35.3	
.03	35.0	95
.04	35.4	
.06	35.2	
.09	35.0	
.10	35.1	
.12	35.3	95
.13	35.5	100
.13½		105
.14	35.4	110

40 ml of $NaHCO_3$ given i.v. at 2.01 p.m.

DERIVED DATA.

PERIOD	1‡	2	3	4	5	6	7	8	9	10	11
Urine Volume (ml/min)	0.99	0.12	0.86	0.56	0.77	0.18	0.56	1.01	1.17	1.80	2.10
Midperiod Serum PAH (mg/100 ml)	6.60	35.6	42.2	50.7	51.9	48.8	49.5	50.6	56.6	63.8	71.0
Creatinine (mg/100 ml)	8.40	5.52	5.84	6.38	6.72	7.10	6.94	7.31	7.15	6.97	7.50
Na (meq/l)	-	-	132.6	130.2	128.2	125.3	126.8	132.4	130.6	128.5	131.5
K (meq/l)	3.13	3.52	3.22	2.88	2.74	2.64	2.89	2.83	2.79	2.75	2.56
Ca (mg/100 ml)	9.0	9.2	9.4	9.6	9.8	10.1	10.4	10.4	10.4		
Mg (mg/100 ml)	1.46	1.59	1.57	1.55	1.55	1.58	1.53	1.71	1.68	1.63	1.42
Inorganic Phosphate (mg/100 ml)	4.0	4.39	4.42	4.42	4.40	4.36	4.36	4.45	4.40	4.35	-
Average Temperature °C	-	29.2	25.3	16.5	12.0	12.2	27.7	34.7	35.2	-	-
Cer (ml/min)	12.4	2.3	13.5	5.9	6.8	1.1	3.9	13.8	17.5	17.7	14.2
CPAH (ml/min)	47.0	6.4	35.5	12.4	13.6	2.6	9.8	34.8	35.5	25.4	20.25
TmPAH (mg/min)	21.5	1.5	9.8	3.5	3.8	0.75	3.1	11.2	10.9	5.8	5.1
% Excretion of filtered:											
Na	-	*1.3	1.1	4.5	7.8	13.0	6.6	0.9	1.1	2.4	2.3
K	20.4	2.4	21.8	28.7	35.2	29.2	46.3	29.8	27.1	28.8	22.0
Ca	4.2	-	2.7	6.8	8.7	12.2	-	5.1	3.4	4.8	•
Mg	9.9	-	2.2	2.3	13.7	-	5.7	2.5	4.9	14.8	26.6
P	3.3	1.9	2.4	2.5	2.8	3.3	4.5	3.2	3.1	3.2	-
H_2O	8.0	5.2	6.4	9.5	11.3	16.4	14.4	7.3	6.7	10.2	14.8

‡ The first blood sample was withdrawn 1½ minutes after the midpoint of period 1. All the figures in column 1 must therefore be taken as approximations.

* The serum concentration of Na in this calculation is that pertaining to serum 2, taken 1½ minutes after the midpoint of period 2.

CHAPTER VIII.

DOG E.

The aims of the experiment on Dog E, were identical to those for Dogs C and D.

Analysis of results obtained so far, suggested that stopping the 'sustaining' infusion of creatinine and PAH at the time of commencement of carddopulmonary bypass, was probably the best method available to us of attaining consistent serum levels of these substances. In consequence, we adopted the same technique here.

Following our observation on the haemorrhagic effects of PAH during surgery (of Dog D) we gave it here only after all major surgery was completed.

In all other respects, the experimental procedure adopted was similar to that for Dog D.

RESULTS:

The results are given graphically in Figs. 15, 16 and 17. Details of experimental and derived data are given at the end of this Chapter.

URINE FLOW RATE; OSMOLALITY; TEMPERATURE; Ccr: (FIG. 15).

During the 20 minute long prebypass observation period, urine flow rate and Ccr both fell, from 6.7 to

3.4 ml/min., and from 61.7 to 22.0 ml/min, respectively. Urinary osmolality fell from 290 to 179 mOsm/l. The oesophageal temperature just before bypass was 33.2°.

The introduction of bypass saw a further marked drop in urine flow rate and Ccr, but now the osmolality rose, to 245 mOsm/l, and within a few minutes to 402 mOsm/l. The mean oesophageal temperature dropped to 28.5° (period 4). The arterial B.P. was 100 mm Hg.

Blood stream cooling was initiated with urine collection period 5. The Cčr rose immediately, from 10.5 to 27.7 ml/min. As cooling progressed, to a low of 11.5° (mean oesophageal temperature) in period 12, urine flow rate increased (to 3.7 ml/min), Ccr fell (to 4.8 ml/min.) and urinary osmolality fell to 241 mOsm/l, (in period 10); urine osmolality then rose slightly to reach 265 mOsm/l in period 12.

On rewarming, the urinary flow rate fell still further, to 3.08 ml/min. and then abruptly to 0.68 ml/min., (periods 13 and 14, respectively). This abrupt drop in urine flow rate occurred at the onset of <u>active</u> rewarming, between periods 13 and 14. Urine flow rate remained at 0.40 to 0.68 ml/min. throughout the remainder of the bypass period.

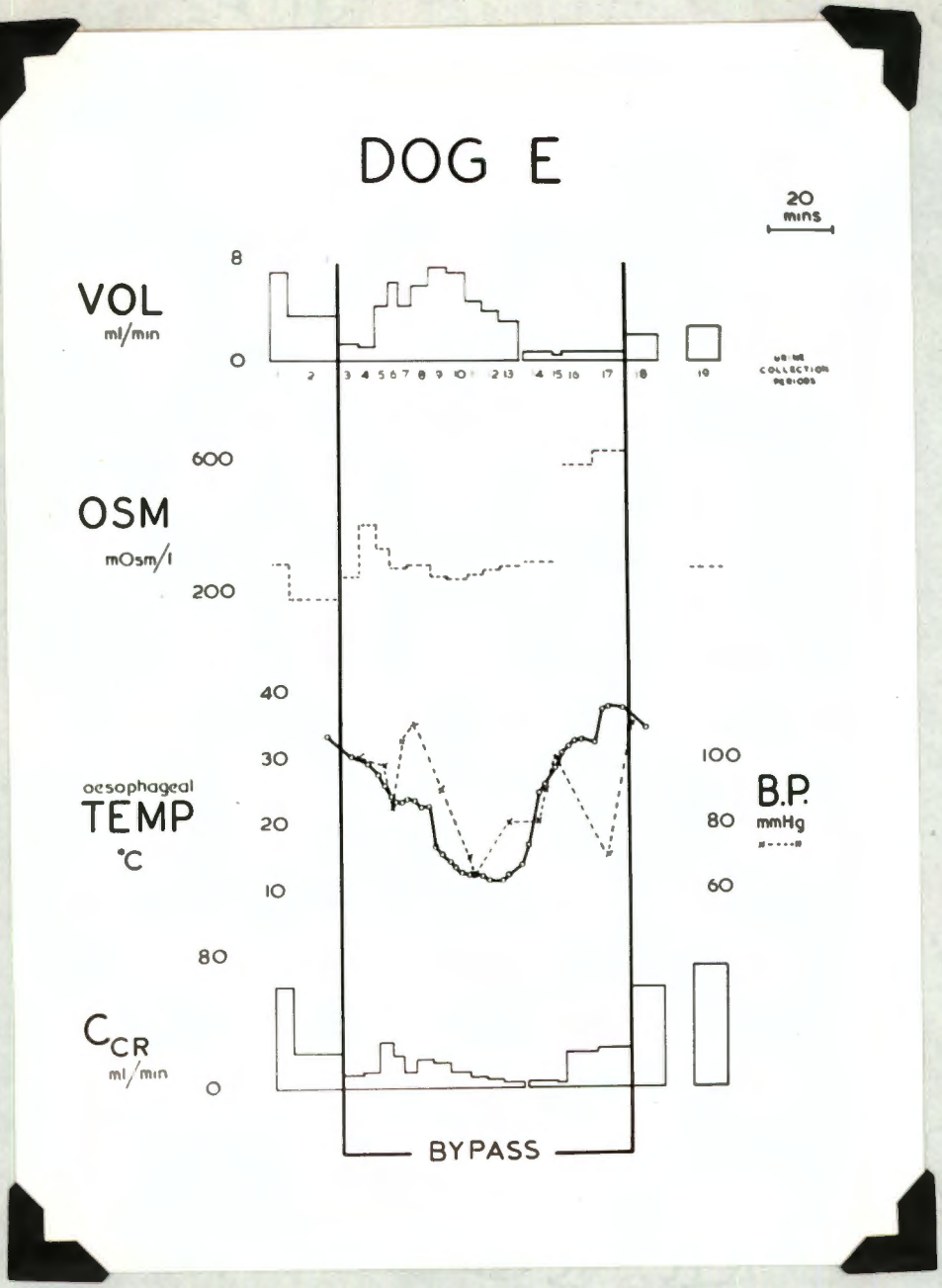

DOG E
20 mins
VOL
ml/min
8
0
OSM
mOsm/l
600
200
oesophageal
TEMP
°C
40
30
20
10
B.P.
mmHg
100
80
60
C_{CR}
ml/min
80
0
BYPASS

FIG. 15.

Ccr fell to 3.8 ml/min. (period 15) but then rose sharply to reach 23.3 ml/min. during period 17.

The arterial systolic blood pressure fluctuated widely during the bypass period. A transient drop from 98 to 85 mm Hg, on commencement of blood stream cooling, was soon reversed to a peak of 110 mm Hg. in period 7. The B.P. then fell again, to a low of 65 mm Hg. in period 11, before rising to 100 in period 14. There followed another drop to 70 mm Hg. in period 17, before a final rise to 110 mm Hg at the end of the bypass period.

Urinary osmolality rose slightly from its low point of 241 mOsm/l. in period 10, to 289 mOsm/l. in period 14. Then followed a steep rise to 582 and 625 mOsm/l. in periods 16 and 17 respectively. Urinary osmolality was not determined for period 15.

Restitution of normal circulation was accompanied by an immediate rise in urine flow rate, (from 0.63 ml/min to 1.90-2.55 ml/min.,) and Ccr (from 23.3 ml/min to 60.9-74.1 ml/min). Urinary osmolality fell to 274 mOsm/l.

URINARY ELECTROLYTE AND PAH CONCENTRATION CHANGES. (FIG. 16)

During prebypass periods 1 and 2, the urine concentrations of Na, K, Ca, inorganic phosphate and PAH all fell, that of PAH relatively more so than the others.

The initiation of bypass produced a rise in concentration of all these substances, except that of PAH, which fell further.

Active blood stream cooling lead to a rise in Na, Ca and PAH concentrations, while the concentrations of inorganic phosphate and K fell. During profound hypothermia Na reached a maximum concentration of 127.2 meq/l., and Ca 8.1 mg/100 ml. These maxima did not co-incide with the period of lowest mean oesophageal temperature (period 12) but with urine collection periods 14 and 13 respectively. K and PAH attained minimal concentrations (of 2.3 meq/l. and .078 G/100 ml respectively) during periods 9 and 11.

On active rewarming, Na and Ca concentrations fell, while those of K, PAH and inorganic phosphate rose (period 16). Period 17 witnessed a reversal, of minor degree, in these changes.

After restitution of normal circulation, urinary

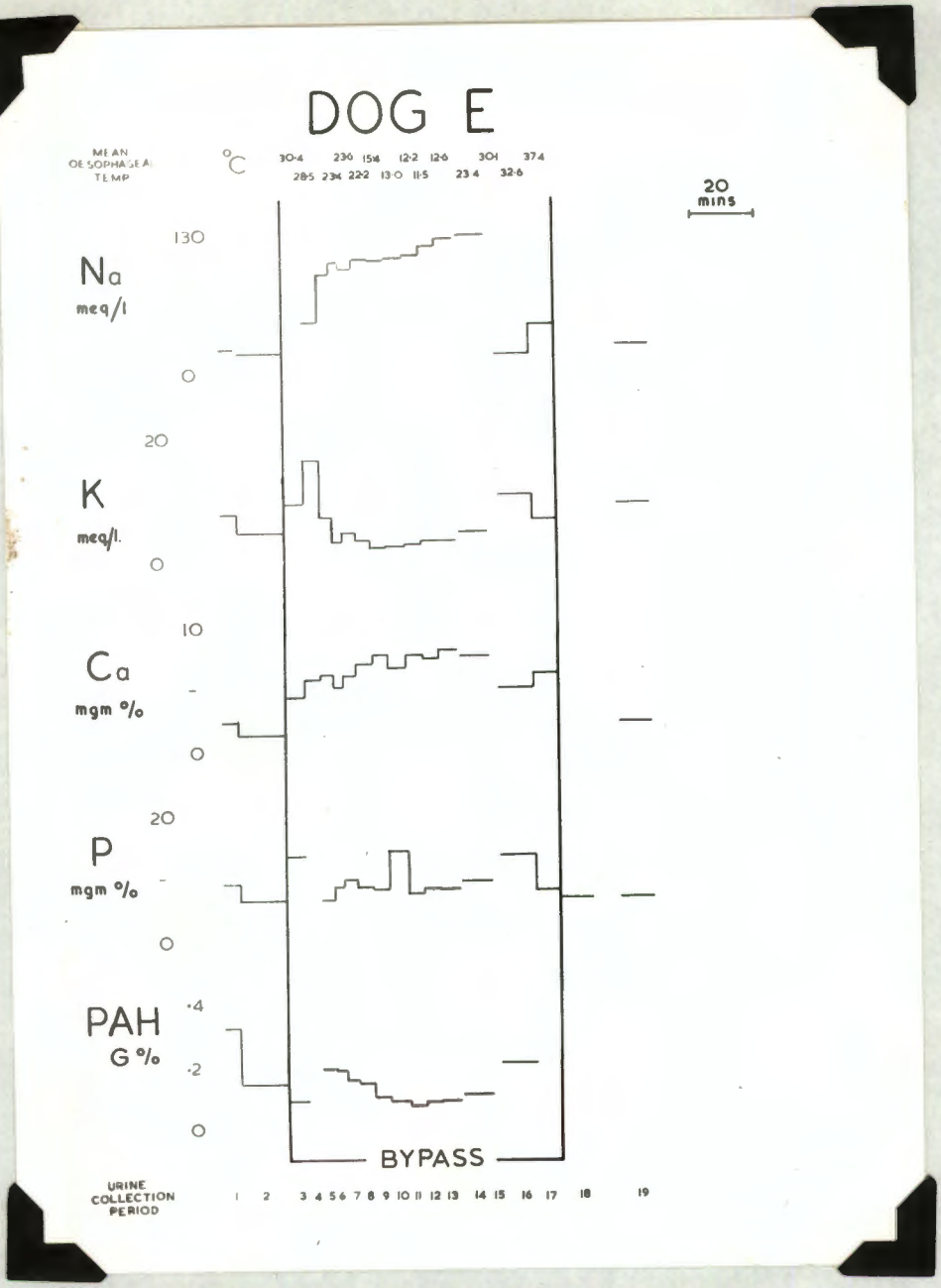

DOG E
MEAN OESOPHAGEAL TEMP
°C
30·4 23·0 15·4 12·2 12·6 30·1 37·4
28·5 23·4 22·2 13·0 11·5 23·4 32·6
20 mins
Na
meq/l
130
0
K
meq/l.
20
0
Ca
mgm %
10
0
P
mgm %
20
0
PAH
G %
·4
·2
0
BYPASS
URINE COLLECTION PERIOD
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

FIG. 16.

electrolyte concentrations were, relatively, little different from those prevailing in period 17. Na and Ca concentrations fell somewhat, inorganic phosphate remained unaltered, and K concentration rose slightly.

PERCENTAGE EXCRETION OF FILTERED WATER AND ELECTROLYTE. (FIG. 17)

The percentage excretion of both Na and water rose, in parallel, with a falling oesophageal temperature. Single curves can be drawn through the data obtained on cooling and rewarming.

Examination of the data for percentage excretion of 'filtered' K, on rewarming, suggests that K excretion falls with a rising oesophageal temperature. The converse does not seem to be true.

SERUM CONCENTRATION CHANGES:

Serum creatinine concentration fell from 5.83-6.25 mg/100 ml before bypass, to 4.58 mg/100 ml 21 minutes after bypass was begun. Three successive serum samples thereafter showed a irregular fall in serum creatinine concentration to 3.50 mg/100 ml 38 minutes later. From serum 9 onwards, the serum creatinine concentration rose steadily, to finally reach 5.84 mg/100 ml at the conclusion of the experiment.

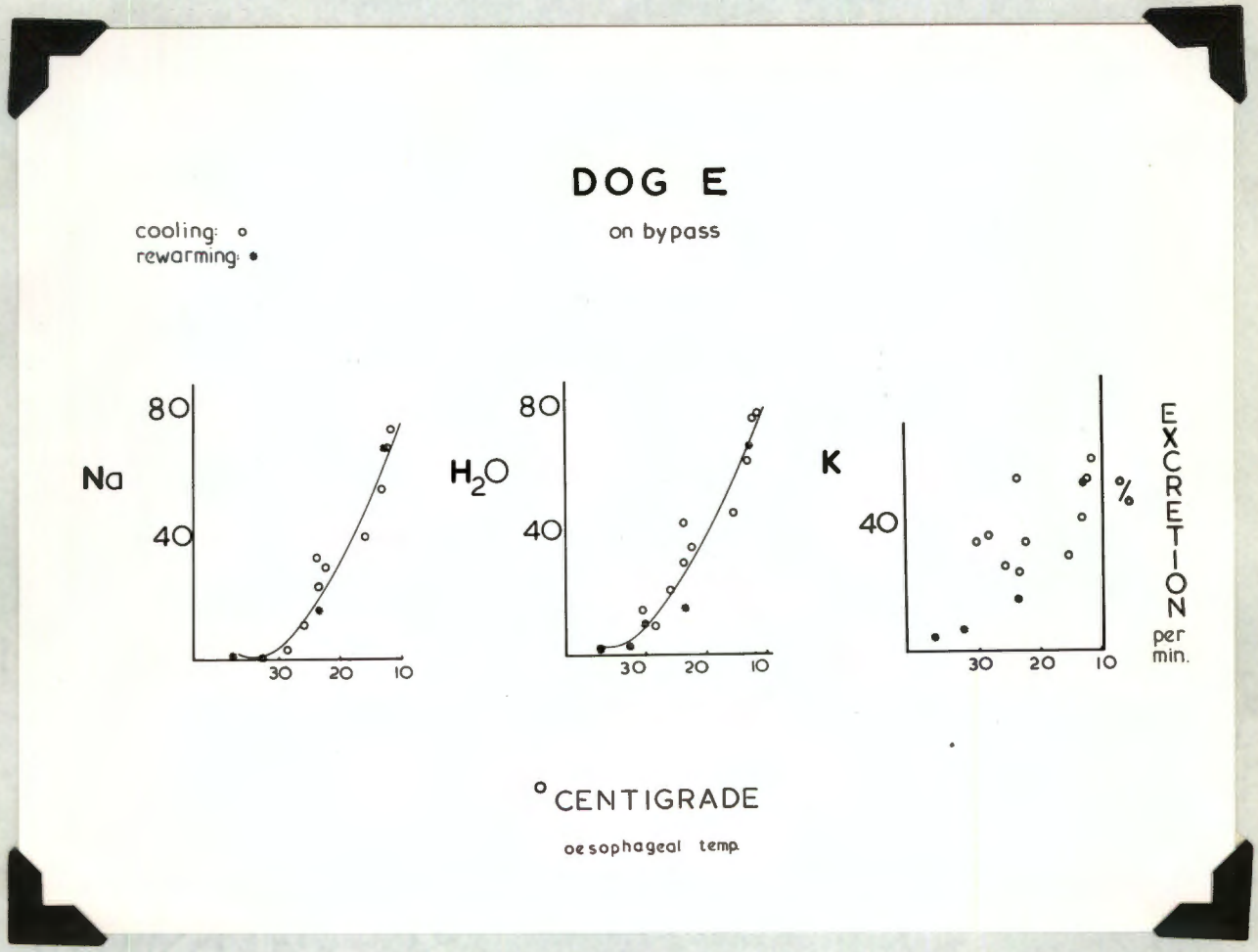

DOG E
cooling: o
rewarming: •
on bypass
Na
80
40
30
20
10
H_2O
80
40
30
20
10
K
40
%
30
20
10
EXCRETION per min.
° CENTIGRADE
oesophageal temp.

FIG. 17.

Na concentration fell from prebypass levels of 143.5 -140.9 meq/l., to a low of 123.5 meq/l. just before bypass was terminated. At the lowest oesophageal temperature (period 12), serum Na concentration was 127.4 meq/l.

The serum K concentration fluctuated irregularly throughout the course of the experiment, varying between 3.10 meq/l to 4.25 meq/l. It rose from 3.50 and 3.10 meq/l to 4.25 meq/l immediately after bypass was begun, then dropped progressively to 3.40 meq/l during period 9, only to rise abruptly to 4.25 to 4.0 meq/l for the remainder of the bypass period. On resumption of normal circulation, the serum K concentration fell again to 3.25-3.15 meq/l.

Only 2 serum samples, No's 1 and 8, were tested for PAH. These contained 41.8 mg/100 ml and 53.6 mg/100 ml respectively.

PROTEINURIA:

The urine was protein free until collection period 14, when active blood stream rewarming began. Proteinuria was first evident as a trace, in urine 14, rose to 10 - 20 mg/100 ml in urine 16, and to a peak of 60-70 mg/100 ml in urine 17. After discontinuation of bypass, it fell, to less than 10 mg/100 ml in the last urine specimen collected. (No.19).

COMMENT:

It will be noticed that the serum samples collected during this experiment were analysed in toto for only Na and K. Creatinine content was estimated in all but two, and PAH, in only two. We had planned to analyse all serum samples for Ca, Mg, inorganic phosphate, and PAH, as in previous experiments. This failure to implement our designs was due, simply and irritatingly, to the fact that the samples were then mislaid.

As a consequence of this, one is unable to clearly gauge the efficacy of our attempt at keeping the serum creatinine level constant. It is apparent from the data, however, that a drop in serum creatinine concentration did occur with the onset of bypass, and that it then fell, slowly and irregularly, until blood No. 8 was drawn during the early rewarming period. The intravenous infusion of creatinine was recommenced some four minutes before blood sample 9 was taken, and it is presumably as a result of this that the serum creatinine levels rose steadily from then onwards.

The abrupt blood pressure fluctuations seen during the bypass period in this dog, were partly artefactual. The arterial systolic blood pressure is well known to fall pari passu with body temperature (cf Chapter 1), a

phenomenon evident in each of our experiments so far. This makes it difficult to evaluate the reasons for the fall in Ccr during hypothermia. We therefore now attempted to keep the systolic blood pressure raised during this period, by increasing the rate of return of (cooled) blood from the extracorporeal circuit to the dog. This effectively increases the animals blood volume, while depleting that of the helix reservoir. The rise in B.P. from 85 to 110 mm Hg. between periods 5 to 7 (mean oesophageal temperatures 25.7 and 23.6° respectively) reflects our use of this manoeuvre. Further cooling, to 12.4°, lowered the B.P. to 65 mm Hg, a figure we could not raise as the helix reservoir was now almost empty. On rewarming, the B.P. rose spontaneously, and about 500 ml. of blood was returned from the dog to the machine. A sharp drop in B.P. occurred shortly before the end of the bypass period; we corrected this by once again infusing Blood from the now repleted helix reservoir, into the dog.

It may be recalled that Bickford and Winton (cf Chapter 1) working with the isolated, perfused kidney, had noted equality of serum and urinary chloride concentrations below 18°. In this dog, urinary Na

concentration reached serum levels for the first time during period 13 (mean oesophageal temperature 12.6°) some 15 minutes after the oesophageal temperature had fallen below 18°. One is made to wonder again, (cf Chapter IV) if kidney temperature is really identical to that of the oesophagus. On the other hand, the minimum urinary creatinine concentration occurred at the time of lwest oesophageal temperature (period 12). Bearing in mind the fact that urinary Na concentration is a measure of distal tubular function, whereas urinary creatinine concentration is partly determined by proximal tubular activity, an alternative explanation presents itself; namely, that temperature gradients exist within the kidney substance itself.

Both infusions - of 5% dextrose-saline, and of the N saline containing the creatinine and PAH - were recommenced at the beginning of urine collection period 16, rather than at the termination of the bypass period, as had been planned. We were dismayed by the sudden marked drop in urine flow rate from period 14 onward, and hoped that the resumption of the infusions would again produce diuresis. The sustaining infusion of creatinine and PAH had perforce to be resumed simultaneously, so as to avoid lowering of serum creatinine and PAH concentrations.

The results show that we did not succeed in producing an immediate diuresis, and that the rate of the creatinine -PAH infusion was too fast.

EXPERIMENTAL DATA ON DOG E.

WEIGHT: 17.3 Kg.

INFUSIONS: 1. 5% dextrose-saline
2. Creatinine 1.5 G)
PAH 2.4 G) in a litre of N saline.

PRIMED: at 11.54 am., with creatinine 0.5G., PAH 1.8G

EQUILIBRATION PERIOD: 30 minutes.

URINE COLLECTION via polyethylene catheter tied into left ureter; catheter tip within renal pelvis.

TIMES OF BLOOD AND URINE COLLECTIONS:

BLOOD SAMPLES:	① 12.24	② 12.43	③ 12.49		④ 12.56		⑤ 1.05		⑥ 1.15		
URINE COLLECTION PERIODS:	12.24 to 12.29 ①	to 12.44 ②	to 12.50 ③	to 12.55 ④	to 12.59 ⑤	to 1.02 ⑥	to 1.06 ⑦	to 1.11 ⑧	to 1.16 ⑨	to 1.22 ⑩	to 1.27 ⑪

BLOOD SAMPLES:	⑦ 1.29			⑧ 1.43		⑨ 1.55	⑩ 2.07	⑪ 2.17		⑫ 2.37	
URINE COLLECTION PERIODS:	to 1.32 ⑫	to 1.38 ⑬		1.39 to 1.48 ⑭	to 1.51 ⑮	to 2.02 ⑯	to 2.10 ⑰	2.20 ⑱		2.29 to 2.39 ⑲	

Cardiopulmonary bypass was begun at 12.44 p.m. bloodstream cooling at 12.55; rewarming at 1.39, and bypass discontinued at 2.10 p.m.

NOTES: (1) Creatinine and PAH sustaining infusion set up and priming dose given, after ureter catheterised and chest opened.

(2) Both infusions were switched off at time of institution of bypass; resumed before bypass discontinued, at 1.51 p.m. i.e. at end of urine collection period 15.

(3) Urine blood-tinged on rewarming.

OBSERVED DATA.

TIME	OESOPHAGEAL TEMPERATURE °C	mmHg Arterial B.P.
12.40	33.2	
47	30.2	
49	30.0	100
52	29.0	
55	27.3	
57	25.8	98
59	24.0	85
1.00	23.3	
02	23.3	105
04	23.7	
.06	23.6	110
.08	22.1	
.10	22.6	
.12	16.1	
.14	15.2	90

TIME	OESOPHAGEAL TEMPERATURE °C	mmHg B.P.
1.16	14.0	
.18	13.2	
.20	12.6	
.22	12.2	70
.24	12.4	65
.26	12.0	
.28	11.6	
.32	11.4	
.34	12.1	80
.38	13.9	
.40	16.7	
.43	24.9	80
.45	26.0	90
.48	28.6	100
.50	30.7	

TIME	OESOPHAGEAL TEMPERATURE °C.	ARTERIAL BLOOD PRESSURE mmHg.
1.52	31.7	
.54	32.4	
.56	32.6	
2.00	32.1	
.02	37.1	
.04	37.5	70
.08	37.4	
2.11		110
.15	34.4	
3.15	36.0	

At 1.43, given 40 ml $NaHCO_3$, intravenously.

URINE

URINE NO.	1	2	3	4	5	6	7	8	9
Volume (ml.)	33.5	51.5	7.4	4.9	21.0	17.8	17.0	29.0	36.0
Osmolality (mOsm/l)	290	179	245	402	334	270	285	284	246
Creatinine (mg/100 ml)	54.2	39.2	41.6	59.2	34.2	16.7	11.0	12.5	8.2
Na(meq/l)	25.0	19.4	-	50.7	95.0	106.6	100.0	110.0	109.4
K (meq/l)	7.8	4.7	9.4	16.3	7.1	3.3	4.8	3.6	2.3
Ca(mg/100 ml)	2.4	1.4	4.4	5.8	6.2	5.1	6.1	7.0	7.6
PAH (G/100 ml)	0.326	.143	.093	-	.192	.186	.158	.146	.102
Inorganic Phosphate (mg/100 ml)	9.05	6.27	13.4	-	6.61	8.45	9.74	8.64	8.18
Protein (mg /100 ml)	o	o	o	•	o	o	o	o	o

Urine No.	10	11	12	13	14	15	16	17	18	19
Volume (ml)	40.0	22.0	18.5	18.5	6.1	1.2	6.9	5.0	19.0	25.5
Osmolality (mOsm/L)	241	252	265	276	289	-	582	625	-	274
Creatinine (mg/100 ml)	5.96	5.00	5.00	5.23	23.2	34.4	127.5	150.0	135.2	162.8
Na (meq/l)	111.2	113.8	121.0	127.2	130.5	-	18.0	47.0	-	27.8
K (meq/l)	2.5	2.9	3.4	3.4	4.9	-	10.6	6.9	-	9.4
Ca (mg/100 ml)	6.6	7.6	7.4	8.1	7.7	-	5.1	6.3	-	2.4
PAH (G/100 ml)	.090	.078	.090	.093	.111	-	.214	-	-	-
Inorganic Phosphate (mg/100 ml)	14.3	7.83	8.35	8.35	9.56	-	13.6	8.0	6.95	6.95
Protein mg/100 ml	o	o	o	o	tr	-	10-20	60-70	-	tr

BLOOD

BLOOD SAMPLE	1	2	3	4	5	6	7	8	9	10	11	12
Creatinine (mg/100 ml)	5.83	6.25	-	-	4.58	3.67	3.83	3.50	3.80	4.10	4.25	5.84
Na (meq/1)	143.5	140.9	137.8	137.8	129.9	127.4	127.4	127.4	124.8	123.5	129.9	133.5
K (meq/1)	3.50	3.10	4.25	4.00	3.60	3.40	4.25	4.25	4.15	4.0	3.25	3.15
PAH (mg/100 ml)	41.8	-	-	-	-	-	-	53.6	-	-	-	-

Plasma Haemoglobin at conclusion of experiment: 228 mg/100 ml

DERIVED DATA.

PERIOD	1	2	3	4	5	6	7	8	9	10
Urine Volume (ml/min)	6.70	3.43	1.22	0.98	4.20	5.93	4.25	5.80	7.20	6.67
Midperiod Serum:										
Creatinine (mg/100 ml)	5.89	6.11	5.95	5.53	5.19	4.92	4.65	4.26	3.81	3.72
Na (meq/l)	143.2	141.8	138.8	137.8	136.9	133.8	130.8	129.0	127.8	127.4
K (meq/l)	3.45	3.24	3.87	4.12	3.96	3.80	3.64	3.53	3.43	3.64
Average Temperature °C	-	-	30.4	28.5	25.7	23.4	23.6	22.2	15.4	13.0
Ccr (ml/min)	61.7	22.0	8.5	10.5	27.7	20.2	10.1	17.0	15.8	10.7
Percentage Excretion of Filtered:										
Na	1.9	2.1	-	3.0	10.5	23.4	32.2	29.1	39.0	54.4
K	24.6	22.6	34.9	36.9	27.2	25.5	55.5	34.8	30.6	42.8
H_2O	10.9	15.6	14.4	9.3	20.8	29.4	42.1	34.1	45.6	62.3

PERIOD	11	12	13	14	15	16	17	18	19
Urine volume (ml/min)	4.40	3.70	3.08	0.68	0.40	0.63	0.63	1.90	2.55
Midperiod Serum:									
Creatinine (mg/100 ml)	3.78	3.82	3.69	3.51	3.66	3.84	4.06	4.22	5.60
Na (meq/l)	127.4	127.4	127.4	127.3	126.0	124.6	123.6	128.6	136.6
K (meq/l)	3.98	4.25	4.25	4.25	4.20	4.13	4.01	3.40	3.16
Average Temperature °C	12.2	11.5	12.6	23.4	30.1	32.6	≠ 37.4	-	-
Cer (ml/min)	5.8	4.8	4.6	4.5	3.8	20.9	23.3	60.9	74.1
Percentage excretion of filtered:									
Na	67.8	73.2	66.9	15.5	-	0.4	1.0	-	0.7
K	55.3	61.7	53.6	17.4	-	7.7	4.7	-	10.2
H_2O	75.9	77.1	67.0	15.1	10.5	3.0	2.7	3.1	3.4

≠ Actual temperature measured for only 6 of the 8 minute period.

CHAPTER IX.

DOG G.

The attempt to minimise or prevent the usual hypothermic B.P. drop, in Dog E, had been at most, only a partial success. We resolved to try again, and as a precautionary measure, primed the helix reservoir with an additional 500 ml. of blood.

We then experimented on Dog F. The aims and technique were identical to those for Dog E. The experiment was a failure, however, for technical reasons, and was abandoned before completion.

Dog G followed. As before, the procedure adopted was similar to that for Dog E. In this dog, to prevent the occurrence of bleeding into the ureter - one of the many complications that had beset us with Dog F - we cannulated the (left) ureter 3 days beforehand.

RESULTS:

The results are given graphically in Figs. 18, 19 and 20. Details of experimental technique, observed and derived data are given at the end of the Chapter.

Urine Flow rate, osmolality, oesophageal temperature, B.P. and Ccr (Fig. 18)

During the 53 minute prebypass period, the urine flow rate fluctuated between 3.0 - 5.4 ml/minute, while the Ccr fell irregularly from 87.4 to 72.6 ml/minute. These inconsistencies were accompanied by variations in urinary osmolality of between 264 to 358 mOsm/l. The arterial systolic B.P. and the oesophageal temperature stayed constant at 130 - 120 mm Hg. and 37°, respectively.

Upon the introduction of cardiopulmonary bypass, urinary flow rate and Ccr fell abruptly to less than half their immediate prebypass values. The oesophageal temperature fell to approximately 30°, and the B.P., after 4 minutes of bypass, to 105 mm Hg. This late fall in B.P. saw a further fall in urinary flow rate and Ccr, and urinary osmolality rose from 298 mOsm/l to 344 mOsm/l.

Active blood stream cooling was initiated at the commencement of urine collection period 7. Therewas an immediate rise in urine flow rate, and a fall in osmolality, but Ccr remained unaltered. As cooling progressed, to a low of 9.2° (period 9), Ccr fell markedly, to 4.9 ml/minute. Osmolality and urinary flow

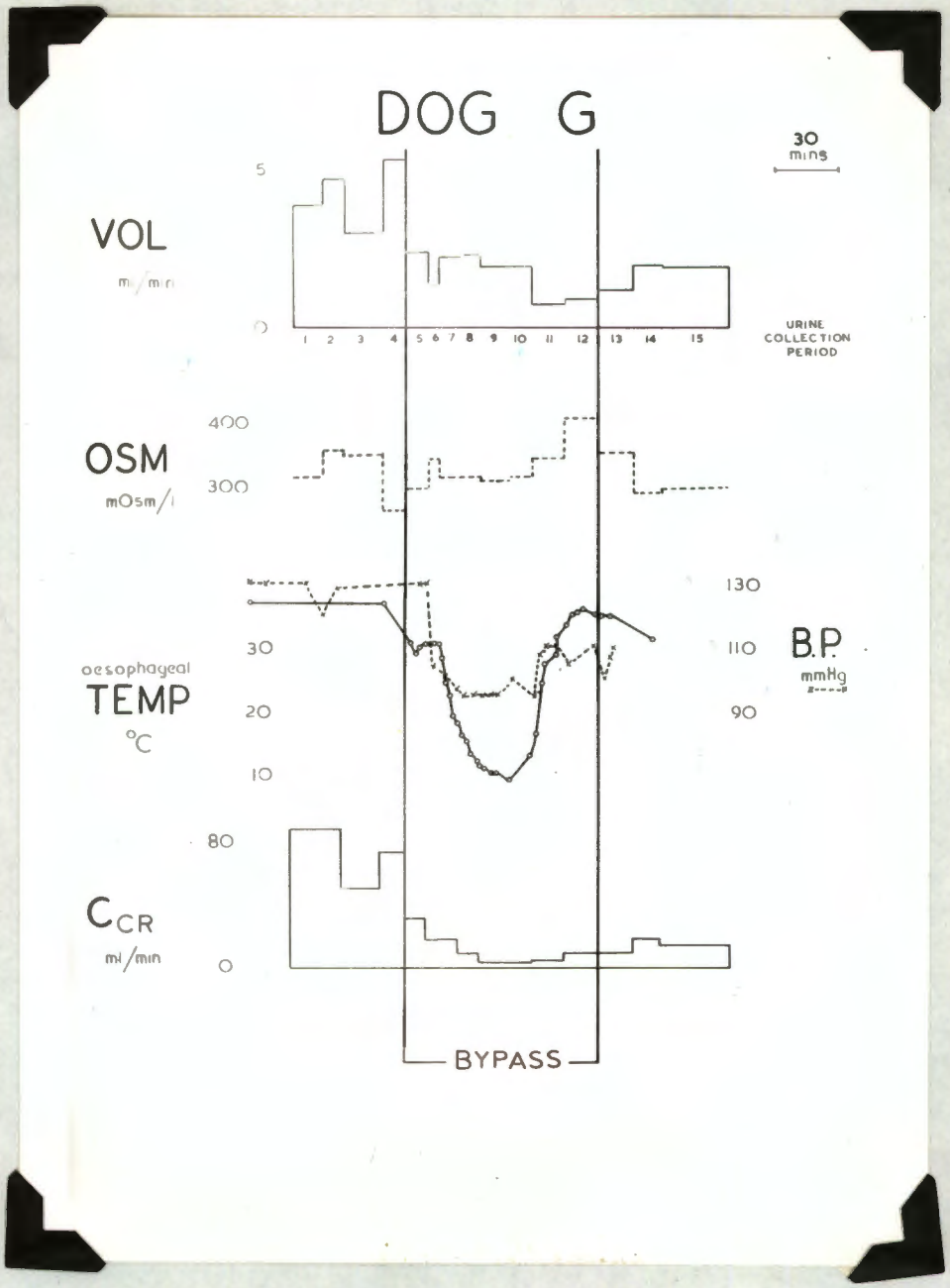

DOG G
30 mins
VOL
5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
URINE COLLECTION PERIOD
OSM
mOsm/l
400
300
oesophageal
TEMP
°C
30
20
10
130
110
90
B.P.
mmHg
C_{CR}
ml/min
80
0
BYPASS

FIG. 18.

rate fell slightly, from 317 mOsm/l to 310 mOsm/l, and from 2.35 to 2.00 ml/minute, respectively. The B.P. was held at 95 mm Hg.

During period 10, active blood stream cooling was stopped, and the oesophageal temperature rose spontaneously to 13°. There was no change in urinary flow rate, osmolality or Ccr.

Active rewarming was begun with urine collection period 11. Urine flow rate dropped immediately, to 0.87 ml/minute, and osmolality rose to 347 mOsm/l. The BP rose to 108 mm Hg, within 3 minutes of rewarming, and the Ccr increased fractionally. As rewarming continued (period 12 - mean oesophageal temperature 35.1°), the urinary flow rate increased, slightly, to 1.03 ml/min, urinary osmolality rose to 408 mOsm/l, and Ccr rose to 10.1 ml/minute.

Restitution of the normal circulation was accompanied by a further rise in urinary flow rate and Ccr, but neither reached prebypass levels. Urinary osmolality fell to 299 mOsm./l. The last blood pressure reading, of 110 mm Hg, was taken 7 minutes after discontinuation of bypass. The oesophageal temperature fell to 35°, 5 minutes after bypass was terminated, and 20 minutes

later had fallen to 31.2°.

URINARY ELECTROLYTE AND PAH CONCENTRATION CHANGES: (FIG. 19)

Prebypass, urine concentrations of Na, K, Mg and Ca fell progressively. Inorganic phosphate and PAH concentrations remained approximately constant.

The introduction of bypass lead to an immediate rise in Na, K, and Ca concentrations. Inorganic phosphate rose fractionally, from 0.81 to 1.00 mg/100 ml. PAH concentration fell, while Mg concentration was unaltered. The Mg content of urine sample 6 was not determined.

Active blood stream cooling lead to a rise in Na, Ca and Mg concentrations, so that by period 9 (mean oesophageal temperature 10.2°), they were 124.0 meq/l., 6.6 mg/100 ml and 1.46 mg/100 ml respectively. K, P and PAH concentrations fell, to 5.1 meq/l., 0.51 mg/100 ml and 24 mg/100 ml respectively.

The dog's temperature was allowed to rise, spontaneously, during period 10 (mean oesophageal temperature 11.9°). Na and Ca concentrations rose further, to 132.5 meq/l. and 6.9 mg/100 ml respectively.

Active rewarming commenced with period 11. The changes seen above, with cooling, all reversed in direction.

Just before termination of bypass (period 12; mean oesophageal temperature 35.1°), urinary concentrations were: Na 10 meq/l; Mg undetectable; Ca 1.0 mg/100 ml. K and PAH concentrations were comparable to those existing just before bypass was initiated. Inorganic phosphate, after rising transitorily to 0.91, fell to 0.51 mg/100 ml.

Postbypass, PAH rose to 2.4 - 1.9 G/100 ml, a higher level than had existed prebypass. Na, Mg and Ca concentrations remained low, while inorganic phosphate fell fractionally. K fell to 3.4 meq/l.

PERCENTAGE EXCRETION OF FILTERED WATER AND ELECTROLYTES. (FIG. 20):

The percentage excretion of filtered Na, H_2O, Mg, Ca and inorganic phosphate, all increased with cooling, and diminished on rewarming. In each instance, close inspection of the data plotted in Fig. 20 suggests that the fall in percentage excretion on rewarming ran parallel to, but slightly higher than, the rise in percentage excretion on cooling. The rate of change of excretion, with cooling, was much less for filtered inorganic phosphate, than for the other electrolytes.

The percentage excretion of filtered K shows a similar relationship to temperature, but too irregularly

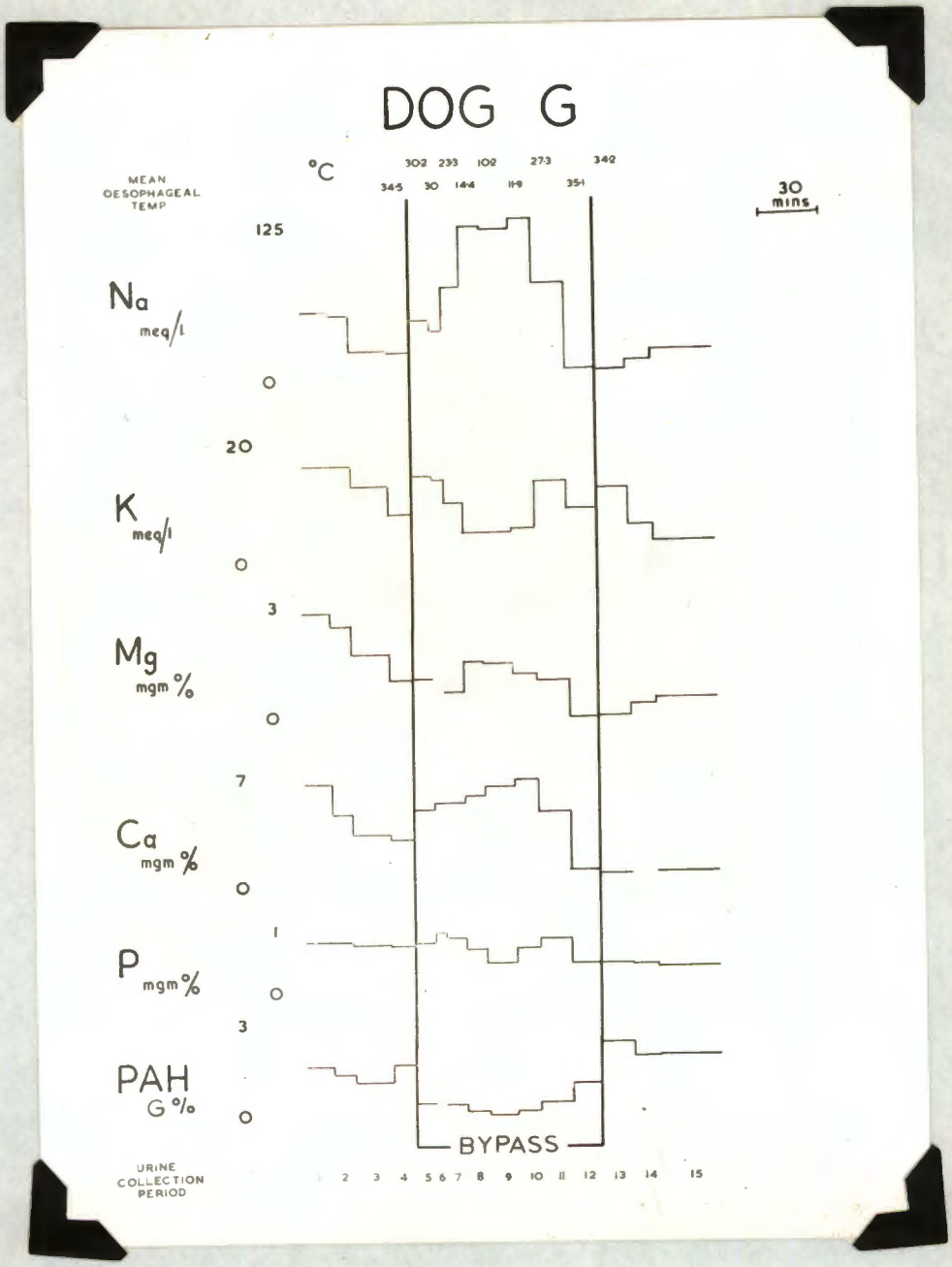

DOG G
MEAN OESOPHAGEAL TEMP
°C
302 233 102 273 342
345 30 144 119 351
30 mins
125
Na meq/l
0
20
K meq/l
0
3
Mg mgm %
0
7
Ca mgm %
0
1
P mgm %
0
3
PAH G %
0
BYPASS
URINE COLLECTION PERIOD
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

FIG. 19.

to permit of close correlation.

SERUM CONCENTRATION CHANGES:

Serum osmolality was 323 - 339 mOsm/l prebypass, fluctuated irregularly between 326 and 352 during the bypass - hypothermic period, and then rose to 355 mOsm/l in the two postbypass serum samples in which it was determined.

During the initial four urine collection periods, serum creatinine fell from 3.70 mg/100 ml to 2.22 mg/100 ml; the introduction of bypass saw a rise to 2.54 mg/100 ml, which continued steadily until serum sample 12, taken 15 minutes after resumption of normal circulation; this had a creatinine content of 7.69 mg/100 ml. The next 2 sera contained 5.18 mg/100 ml and 6.59 mg/100 ml of creatinine.

PAH varied grossly throughout the experiment. Before bypass, it fell from 204 mg/100 ml to 53 mg/100 ml; from periods 5 to 11, it fluctuated between 50.5 mg/100 ml. and 65.8 mg/100 ml; the serum sample taken at the end of period 12, (and at the conclusion of bypass) contained 202 mg/100 ml. The three postbypass serum samples had PAH contents of 165, 139, and 164 mg/100 ml.

Serum Na rose from 125 meq/l to 144.5 meq/l in

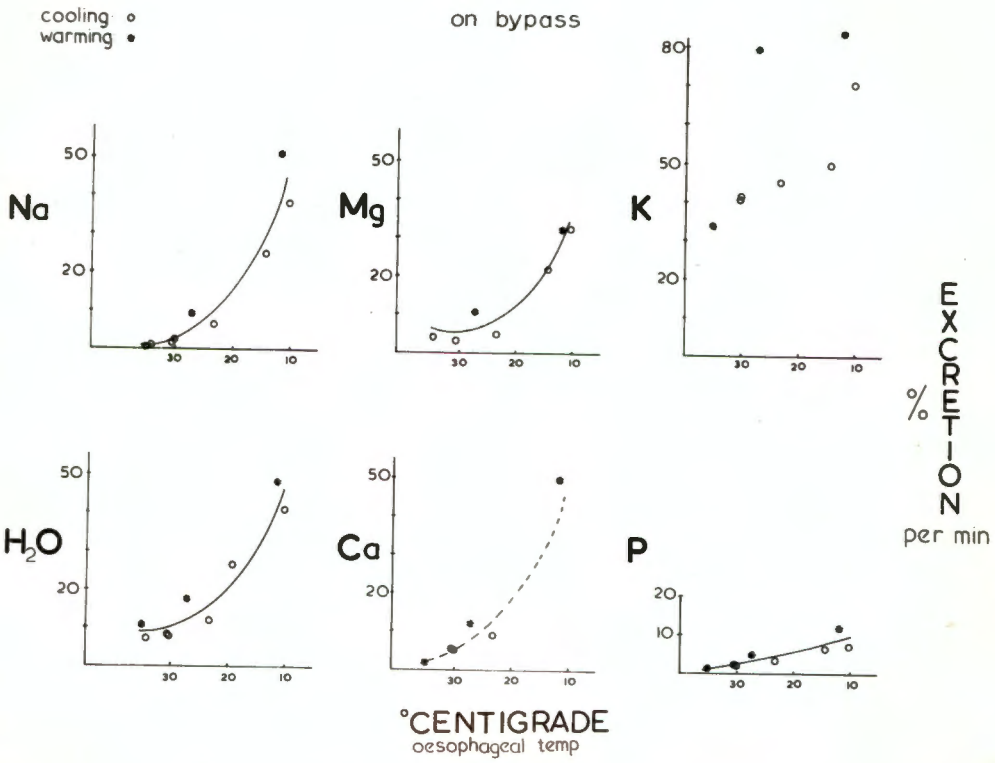

DOG G
cooling ○
warming ●
on bypass
Na
Mg
K
H2O
Ca
P
50
20
80
10
30
20
10
% EXCRETION per min
°CENTIGRADE
oesophageal temp

FIG. 20.

samples 3 and 4. During the bypass-hypothermic period it fell smoothly to 127 meq/l., and was 128 meq/l in sample 11. After rising to 131.5 meq/l fifteen minutes after termination of bypass, it then fell to 121.5 meq/l and finally to 116.5 meq/l.

After an initial reading of 3.89 meq/l, the serum K varied irregularly between 3.20 meq/l and 2.63 meq/l during the remainder of the experiment.

Serum Ca fell from 7.8 mg/100 ml. prebypass, to 6.4 mg/100 ml (serum sample 11), then rose to 7.2 mg/100 ml in the postbypass period.

The serum Mg concentration fluctuated irregularly between 1.61 mg/100 ml and 1.85 mg/100 ml throughout the experimental period.

The inorganic phosphate concentration fell progressively, from an initial 5.24 mg/100 ml, to 1.45 mg/100 ml postbypass.

PROTEINURIA:

The urine was protein-free, until the cessation of active blood stream cooling. Spontaneous rewarming occurred in period 10, the mean oesophageal temperature rising from 10.2° (in period 9) to 11.9°, and a trace

of protein was now detected in the urine. Active rewarming was accompanied by a rise in urine protein content, to 10-20 mg/100 ml, at which level it persisted for the remainder of the experiment.

COMMENT:

This experiment was marred by a serious technical inadequacy. During the 53 minutes of 'control' urine-collection periods 1 to 4, an air bubble was seen partially obstructing the drip apparatus leading from the vacolitre containing the sustaining infusion of creatinine and PAH. The bubble was removed, and the drip rate readjusted, some 20 minutes before cardiopulmonary bypass was begun. The effects of this obstruction, and its subsequent release, are only too evident in the gross irregularities in prebypass serum creatinine and PAH concentrations.

When bypass was begun, the infusions were not stopped, but just slowed down. This departure from previous practice was based on our observations on Dog E, where the serum creatinine concentration was seen to fall during the bypass period, following such stoppage. The drip rates were then speeded up again toward the end of the bypass period (urine collection period 12). Unfortunately, we overshot the mark - the point has been

made several times already that there is no way of knowing the optimum drip rate during bypass - and serum creatinine and PAH concentrations skyrocketted.

The net effect of the above, is that calculations of TmPAH are quite valueless for both the pre- and post- bypass periods. Fortunately, the serum creatinine did not vary quite as much as did the PAH, and so the calculated creatinine clearances are probably meaningfull, particularly during the bypass period itself.

The rise in serum osmolality, fall in serum Na concentration, and increased rate of fall of serum inorganic phosphate content, in the postbypass period, are presumably a reflection of the too - rapid infusion of 10% dextrose water at this time.

In previous experiments, we had often seen some bleeding into the ureter, following catheterisation, but until our experience with Dog F, this had always cleared before collection of the first urine specimens. Knowing we were going to use a large dose of PAH in Dog G, and mindful of its haemorrhagic effect (cf Dog B) we catheterised the (left) ureter in Dog G 3 days beforehand. Cursory comparison of the urinary findings here, with those of previous experiments, reveals that this variation in procedure produced no ill effects.

A week later, we experimented on Dog H, again catheterising one ureter (two) days beforehand. The urinary flow rate was unexpectedly slow (1 ml/minute) despite a rapid rate of intravenous infusion of 10% dextrose-water, and when bypass was commenced, it fell to zero. On exploring the wound in the flank, from which the ureteric catheter emerged, a deep pocket of thick pus was found, surrounding the kidney. The experiment was then abandoned. This gross infection had occurred despite the use of antibiotics, and we therefore never again adopted the procedure of preliminary ureteric catheterisation.

EXPERIMENTAL DATA ON DOG G.

WEIGHT: 17.0 Kg

INFUSIONS: (1) 10% Dextrose water
(2) Creatinine 1.5 G)
PAH 2.4G) in a litre of N saline

PRIMED at 10.23 a.m., with creatinine 0.5G, PAH 1.8G

EQUILIBRATION PERIOD: 29 minutes

URINE COLLECTION: via polyethylene catheter tied into left ureter; catheter tip in renal pelvis

TIMES OF BLOOD AND URINE COLLECTIONS.

BLOOD SAMPLES:	(1) 10.59	(2) 11.13	(3) 11.31			(4) 11.45	(5) 11.57	(6) 12.05	(7) 12.15	(8) 12.31	(9) 12.40	(10) 12.59	
Urine collection periods:	10.52 to 11.06 (1)	to 11.15 (2)	to 11.34 (3)	to 11.45 (4)		11.46 to 11.55 (5)	to 12.0 (6)	to 12.10 (7)	to 12.20 (8)	to 12.35 (9)	to 12.45 (10)	to 1.00 (11)	to 1.15 (12)

BLOOD SAMPLES:	(11) 1.15	(12) 1.23	(13) 1.44	(14) 2.16
Urine collection periods:	to 1.31 (13)	to 1.45 (14)	to 2.16 (15)	

Cardiopulmonary bypass was begun at 11.45 a.m., active blood stream cooling at 12.00 noon; rewarming at 12.45; and bypass discontinued at 1.15 p.m.

Notes: Ureter catheterised 3 days previously.

OBSERVED DATA.

URINE								
Urine No.	1	2	3	4	5	6	7	8
Volume (ml)	54.0	42.5	57.0	59.0	22.0	7.0	23.5	24.0
Osmolality (mOsm/L)	316	358	348	264	298	344	317	316
Creatinine (mg/100 ml)	83.8	67.9	54.6	34.6	30.0	33.1	25.4	11.5
Na (meq/l)	54.9	53.0	24.5	22.1	48.6	40.0	76.3	126.3
K (meq/l)	16.0	16.0	12.5	7.9	14.2	13.7	10.0	5.1
Ca (mg/100 ml)	6.6	4.8	3.3	3.0	5.1	5.5	5.5	6.0
Mg (mg/100 ml)	2.73	2.48	1.66	0.99	1.09	-	0.72	1.49
PAH (G/100 ml)	1.677	1.395	1.135	1.686	0.478	-	0.354	0.153
Inorganic Phosphate (mg/100 ml)	0.87	0.87	.80	.81	.84	1.00	.90	.73
Protein (mg/100 ml)	o	o	o	o	o	o	o	o

Urine No.	9	10	11	12	13	14	15
Volume (ml)	30.0	20.0	13.0	14.0	22.0	30.0	66.0
Osmolality (mOsm/l)	310	318	347	408	351	291	299
Creatinine (mg/100 ml)	8.4	7.6	21.9	53.0	59.6	53.8	42.0
Na (meq/l)	124.0	132.5	80.0	10.0	8.0	16.0	25.0
K (meg/l)	5.1	5.5	13.5	8.8	11.2	6.5	3.4
Ca (mg/100 ml)	616	6.9	4.8	1.0	0.8	-	0.9
Mg (mg/100 ml)	1.46	1.20	1.10	0.00	0.15	0.44	0.51
PAH (G/100 ml)	0.024	0.158	0.435	1.109	2.401	1.925	1.925
Inorganic Phosphate (mg/100 ml)	.51	.75	.91	.51	.51	.48	.46
Protein (mg/100 ml)	o	tr	10-20	10-20	10-20	10-20	10-20

BLOOD

Blood Sample	1	2	3	4	5	6	7
Osmolality (mOsm/L)	-	323	-	339	339	326	329
Creatinine (mg/100 ml)	3.70	3.70	3.07	2.22	2.54	3.03	3.03
Na (meq/l)	-	-	125.0	144.5	145.0	137.5	135.0
K (meq/l)	3.89	-	2.95	-	2.63	-	2.70
Ca (mg/100 ml)	-	-	7.8	-	7.5	-	7.6
Mg (mg/100 ml)	-	1.63	-	1.76	1.61	1.70	1.71
PAH (mg/100 ml)	204	124	84.3	53.0	58.3	50.5	61.6
Inorganic Phosphate (mg/100 ml)	5.24	-	4.78	-	3.50	3.36	3.07

BLOOD SAMPLE	8	9	10	11	12	13	14
Osmolality (mOsm/l)	352	330	336	-	355	-	355
Creatinine (mg/100 ml)	3.53	3.65	3.62	7.02	7.69	5.18	6.59
Na (meq/l)	134.5	127.0	127.0	128.0	131.5	121.5	116.5
K (meq/l)	-	3.20	-	2.63	-	-	-
Ca (mg/100 ml)	-	6.8	-	6.4	-	7.2	
Mg (mg/100 ml)	1.67	1.85	1.64	-	-	-	1.77
PAH (mg/100 ml)	52.1	65.8	60.6	202	165	139	164
Inorganic Phosphate (mg/100 ml)	3.21	3.16	-	3.22	2.17	-	1.45

Plasma haemoglobin at conclusion of experiment: 87 mg/100 ml.

Time	Oesophageal Temperature °C	Arterial Systolic B.P. mmHg.
10.32	37.0	130
.40		130
.59		130
11.07		120
11.13		129
11.35	37.0	
.48	30.5	
.50	29.0 ∓	
.52	30.0	130
.54	30.5	130
.56	30.5	
.58	30.4	105
12.00	30.3	
.02	28.0	
.04	24.2	100
.06	22.2	
.08	19.0	
.10	18.0	96
.12	16.4	95
.14	15.5	
.16	13.5	95
.18	12.2	
.20	11.8	95
.22	11.2	
.24	10.8	95

Time	Oesophageal Temperature °C	Arterial Systolic B.P. mmHg.
12.26	10.2	
.28	10.1	95
.34	9.2	100
.43	13.0	
.45	"	95
.46	16.8	
.48	24.4	108
.50	27.2	110
.55	28.8	110
.56	31.8	
1.00	33.8	105
.02	35.0	
.04	35.6	
.06	35.8	
.08	36.0	
.14	35.4	110
.16	35.0	
.18	-	100
.20	35.0	105
1.22	-	110
.40	31.2	

∓ :as inadvertently began bypass with active cooling; this was corrected immediately, and the active blood stream cooling discontinued.

DERIVED DATA.

Period	1	2	3	4	5	6	7	8
Urine volume (ml/min)	3.86	4.72	3.00	5.36	2.45	1.40	2.35	2.40
Midperiod Serum:								
Creatinine (mg/100 ml)	3.70	3.70	3.30	2.55	2.37	2.57	3.03	3.03
Na (meq/l)	-	-	-	136.8	144.7	144.5	137.5	135.0
K (meq/l)	3.89	3.55	3.14	2.85	2.71	2.63	2.66	2.70
Ca (mg/100 ml)	-	-	-	7.71	7.57	7.49	7.46	7.55
Mg (mg/100 ml)	-	-	1.68	1.74	1.69	1.61	1.70	1.71
PAH (mg/100 ml)	204	138.3	98.7	65.2	55.7	57.8	50.5	61.6
Inorganic Phosphate (mg/100 ml)	5.24	5.07	4.87	4.36	3.82	3.41	3.36	3.07
Average Temperature °C	-	-	-	34.5	30.2	30.4	23.3	14.4
C_{PAH} (ml/min)	-	-	-	-	21.4	-	16.5	6.0
Ccr (ml/min)	87.4	86.6	49.7	72.6	31.1	18.1	19.8	9.1
% Excretion of filtered:								
Na	-	-	-	1.2	2.7	2.2	6.6	24.6
K	-	-	-	18.1	41.3	40.4	44.7	49.3
Ca	-	-	-	2.9	5.3	5.7	8.8	-
Mg	-	-	6.0	4.2	-	3.5	5.1	22.9
P	0.8	1.0	1.0	1.4	1.8	2.3	3.2	6.3
H_2O	4.5	5.5	6.1	7.4	7.9	7.8	11.9	26.3

Period	9	10	11	12	13	14	15
Urine Volume (ml/min)	2.00	2.00	0.87	2.03	1.38	2.14	2.13
Midperiod Serum:							
Creatinine (mg/100 ml)	3.42	3.70	3.65	5.43	7.70	5.90	5.91
Na (meq/l)	134.6	127.0	127.0	127.7	131.5	124.4	118.9
K (meq/l)	2.95	3.20	2.83	2.75	-	-	-
Ca (mg/100 ml)	7.19	6.83	6.68	6.49	6.62	7.03	-
Mg (mg/100 ml)	1.68	1.85	1.71	1.65	1.68	1.69	1.74
PAH (mg/100 ml)	54.2	65.8	62.4	135.8	165.1	146.5	152
Inorganic Phosphate (mg/100 ml)	2.96	3.16	3.18	3.11	2.17	1.97	1.66
Average Temperature °C	10.2	11.9	27.3	35.1	34.2	-	-
C_{PAH} (ml/min)	0.9	4.8	6.1	-	-	-	-
Ccr (ml/min)	4.9	4.1	5.2	10.1	10.6	19.5	15.2
% Excretion of filtered:							
Na	37.5	50.8	9.9	0.8	0.8	1.4	2.3
K	70.3	83.5	79.3	32.7	-	-	-
Ca	-	49.3	12.1	1.7	2.6	-	-
Mg	35.3	31.5	10.7	-	1.2	2.9	4.2
P	7.0	11.6	4.8	1.7	3.1	2.7	3.9
H_2O	40.7	48.6	16.6	10.2	13.0	11.0	14.1

CHAPTER X.

APPARENT ACTIVATION ENERGY.

We had by now experimented on 10 dogs, two of which (Dogs F and H) had been abandoned as failures. Many hundreds of chemical estimations were performed on the scores of blood and urine samples collected. The time was ripe for a detailed examination of our results.

The discussion and interpretation of our data will however be deferred to later Chapters. Only some thoughts pertaining to apparent activation energies will be presented here, as these directly influenced our subsequent studies on renal function during profound hypothermia.

The graphic plot of the logarithm of a reaction, against the reciprocal of the absolute temperature, yields a curve, the slope of which equals the apparent activation energy (cf Appendix E). One wondered whether such manipulation of our data (on the rates of renal reabsorption of the various electrolytes at different temperatures) would also yield smooth curves, or whether they were in fact so crudely obtained as not to be susceptible to this form of analysis.

Inasmuch as the GFR (and therefore the filtered load) varies markedly during cooling and rewarming, the absolute rates of solute reabsorption, at different temperatures, are obviously non-comparable. The actual amount of solute reabsorbed per unit time must therefore be 'corrected' for variations in load; one way of doing this is to express the amount reabsorbed as a fraction of that filtered, per minute.

Previous Chapters contain figures on calculated percentage excretion of solute filtered per minute, at various mean oesophageal temperatures. Subtracting percentage excretion from 100 gives the percentage reabsorption of filtered solute per minute. Omission of the percentage symbol converts this to the resultant of the ratio: amount reabsorbed/amount filtered, per minute. This figure can be regarded as the 'corrected' absolute reabsorption rate, or more elegantly, as the equivalent of the reaction rate constant (or equilibrium constant) in a chemical reaction at a given temperature. (This assumes of course that the reaction rates of renal reabsorption mechanisms are in fact constant for any given temperature, a not unreasonable assumption in view of the demonstration of such constancy for a host of enzyme mediated reactions in other biological systems (84).

Cardiopulmonary bypass in itself alters the percentage of filtered solute that is excreted - and therefore the percentage reabsorbed - per minute (cf Chapter V). Clearly therefore only those data pertaining to the bypass period can be utilised in the evaluation of the additional effects of temperature on renal reabsorption rates.

In the experiment on Dog B, we had collected a number of urine samples during the total bypass period, at mean oesophageal temperatures varying between 37.7° and 11.9°. Fig. 21 shows the plot of the logarithms of the 'reaction rate constants' (i.e. the ratios of reabsorbed to filtered solute, per minute) against the reciprocals of those absolute temperatures at which these rates were measured, for Na, Ca, inorganic phosphate, and Mg. It is apparent that curves can readily be drawn to these data.

This ability to obtain a smooth curve on an Arrhenius plot of our reabsorption data, is not perhaps surprising. All the experiments in which hypothermia was induced (with the exception of Dog D) showed a curvilinear relationship between the percentage excretion of solute filtered per minute, and the oesophageal temperature, expressed in degrees centigrade. As the logarithm of the 'corrected'

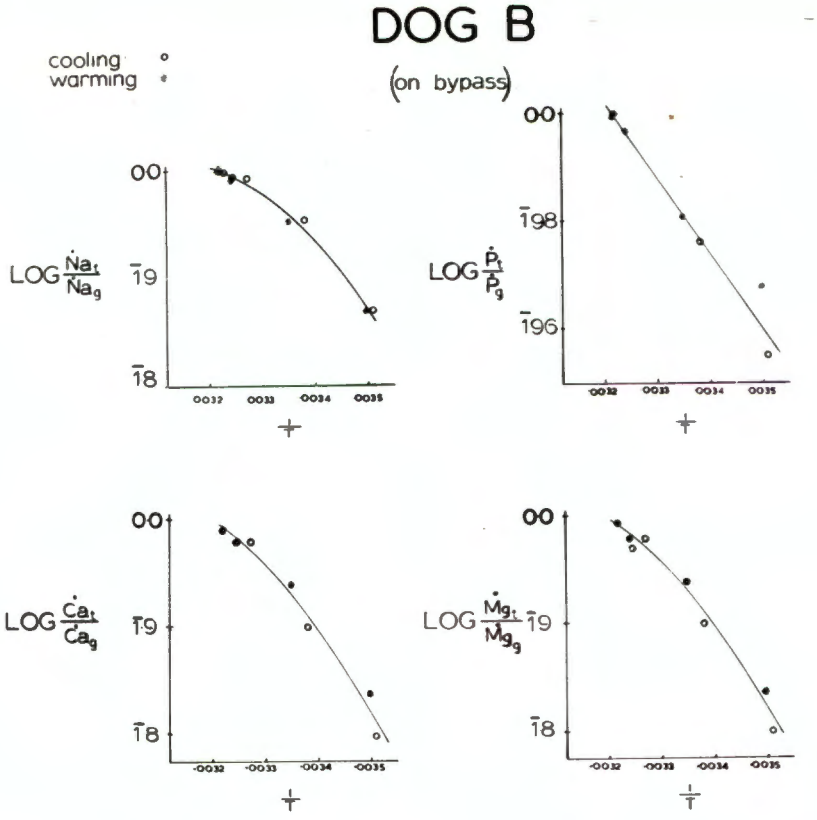


FIG. 21

Legend: Dog B. The Arrhenius plot of 'reaction rate constants' against temperature, for the renal reabsorption of Na, Ca, P and Mg.

$\frac{\dot{Na}_t}{\dot{Na}_g}$ = the rate at which Na is reabsorbed by the tubules, divided by the rate at which Na is filtered through the glomeruli.

(A dot placed over the symbol of a substance, to denote rate of a reaction, is a form of shorthand first used by Newton)

reabsorption rate is directly derived from the former, and as the numerical relationship of the latter to the Absolute temperature is almost constant over so small a temperature range, it may in general be expected that a similar degree of correlation will be found on an Arrhenius plot based on the same data.

It will be recalled that the temperature was lowered 'step-wise' in Dog C, in an endeavour to ensure that the urinary findings observed during a temperature plateau were in fact characteristic of that temperature, and not simply the resultant of changes which might have occurred over a wide range of oesophageal temperatures, as in Dog B. Yet the regression line for the percentage excretion of filtered solute per minute, versus oesophageal temperature, for Dog C, is less clearly delineated than that for Dog B. The Arrhenius plot (not shown) is similarly somewhat irregular. Inspection of Fig. 11 shows that only urine collection periods 7, 8, 12, 13 and 17 were taken at times of a fairly constant oesophageal temperature; consequently data obtained for these periods are probably the most accurate. Fig. 22 shows the Arrhenius plot of the reabsorption rates for Na, Ca, Mg and inorganic phosphate, based on the data from these urine collection periods. The regression lines for Na and particularly Mg, are seen to be not too clearly defined, and were therefore positioned by inspection.

DOG C

on bypass

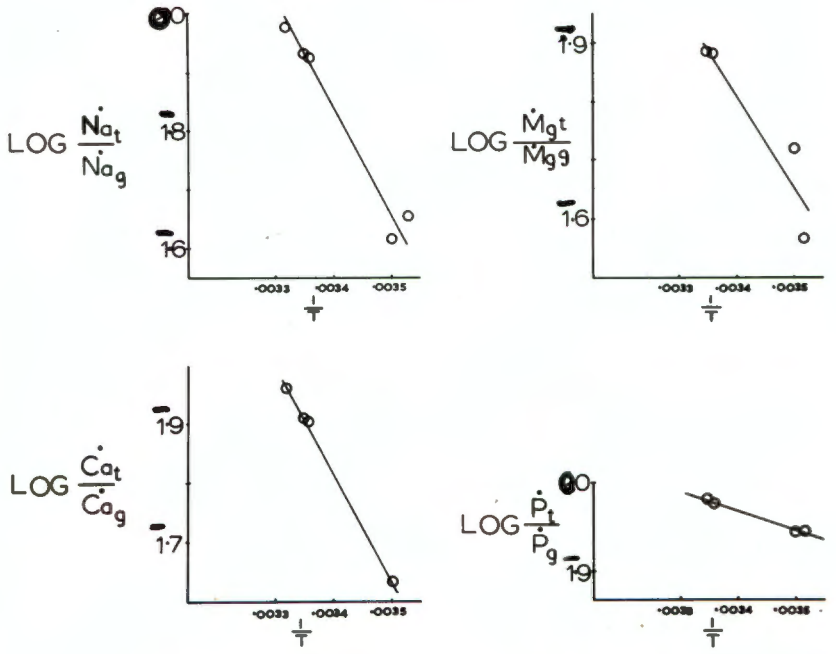


FIG. 22.

The apparent activation energy can be derived from the formula:

$$\log\left(\frac{K1}{K2}\right) = \frac{E\ (T_1 - T_2)}{2.3\ R\ (T_1 . T_2)}$$ where k1 and k_2 are the reaction rate constants at absolute temperatures T_1 and T_2; R is the gas constant; and E is the apparent activation energy. Our data were too variable to apply this equation directly. To calculate E, therefore, for any solute, my procedure was to note which urine collection samples embraced the periods of least variation in oesophageal temperature; plot the data derived from these periods on an Arrhenius graph; draw (by inspection) the regression line with the best fit; and then obtain convenient values of k_1, k_2, T_1 and T_2 from this line, to substitute in the above formula.

No actual example will be given, as the calculated apparent activation energy for the reabsorption of any given solute varied widely from dog to dog. Details will be given in a later Chapter. Suffice it to say that the variability of these results led me to query an axiom with which we had begun this work, viz. Severinghaus' dictum that "organs with high perfusion such as brain or kidney maybe assumed to follow the heart temperature passively", and that heart temperature, in turn, "approximated almost exactly

to the lower oesophageal temperature" (cf Chapter 11). Clearly, if this assumption is false, then calculations of activation energies of renal mechanisms, based on oesophageal temperatures, must give unreliable results.

CHAPTER XI.

DOG I.

Some degree of non-parallelism between changes in oesophageal temperature, and alterations in concurrent percentage excretion of filtered electrolyte, (cf comment on Dogs B and E) had made us doubt the validity of the oesophageal, as a measure of renal, temperature. The wide discrepancies found (in Dogs B to G) in the calculated activation energies for renal reabsorption of the various solutes (cf Chapter X) raised this question anew, and we determined to solve it by direct observation.

A complete Arrhenius plot of reaction rates about an optimum temperature, for any enzyme activated system, may be expected to yield an approximately sinusoidal curve (cf Appendix E). The Arrhenius plots for Na, Ca, and Mg reabsorption in Dog B (Fig. 21) suggest the beginnings of such curves. Knowledge of the slope of descent of reaction rate from, as well as of the slope of ascent toward, the reaction rate at optimum temperature, makes possible the calculation of $\Delta F^{\ddagger}$ and $\Delta S^{\ddagger}$, as well as $\Delta H^{\ddagger}$ (84) We therefore decided to observe renal function, not only during cooling, but also, subsequently, during hyperthermia.

We had, then, two objectives in experimenting on Dog I. The first was to establish the validity or otherwise of the oesophageal, as a measure of the renal, temperature. The second, to obtain data on the percentage excretion of filtered solutes during both hypo- and hyperthermia.

The experimental procedure was similar to that adopted previously, with the following exceptions: (1) after catheterisation of one ureter, a thermistor probe was inserted into the substance of the ipsilateral kidney (2) a much smaller dose of PAH was given than hitherto in order to follow changes in ERPF rather than TmPAH, and (3) after cooling, the dog was rewarmed to above the normal body temperature.

RESULTS:

The results are presented graphically in Fig. 22. Details of the experimental procedure, observed and derived data are given at the end of this Chapter.

URINE FLOW RATE AND OSMOLALITY; RENAL AND OESOPHAGEAL TEMPERATURES; SYSTOLIC ARTERIAL BLOOD PRESSURE; Ccr AND ERPF: (Fig. 23).

During the prebypass urine collection periods 1 to 3, urine flow rate increased from 2.04 ml/min to 3.42 ml/minute, and urinary osmolality fell from 275 mOsm/l. to 198 mOsm/l. This diuresis was accompanied by a slight fall in systolic arterial blood pressure (from 120 mm Hg to 90 mm Hg), and ERPF (91.2 to 75.1 ml/minute) while Ccr fell from 27.1 to

13.2 ml/minute.

At the commencement of cardiopulmonary bypass, both kidney and oesophageal temperatures were 35.0°. Urine flow rate and Ccr fell abruptly to, and remained at, near zero, although the systolic B.P. rose slightly, to 100-105 mm Hg.

Active blood stream cooling was introduced with period 5. There was an immediate rise in urine flow rate (to 1.36 ml/minute), and Ccr (to 10.5 ml/minute). The ERPF was 22.1 ml/minute. As cooling progressed to a low of 15.4° (renal temperature) in period 14, the urinary flow rate after transiently increasing, fell to low values, as did the B.P., Ccr and ERPF.

During urine collection period 8, a transient abrupt rise in systolic B.P, (to 140 mm Hg) occurred, following on accidental increase in perfusion flow rate. This was accompanied by a sharp rise in urine flow rate and Ccr; the ERPF rose slightly too. The mean renal temperature fell from 24.5 to 20.7° at this time.

Comparison of renal and oesophageal temperatures reveals that initially the kidney cooled more rapidly than did the oesophagus. They then both reached much the same temperature during periods 6 and 7, when an attempt was

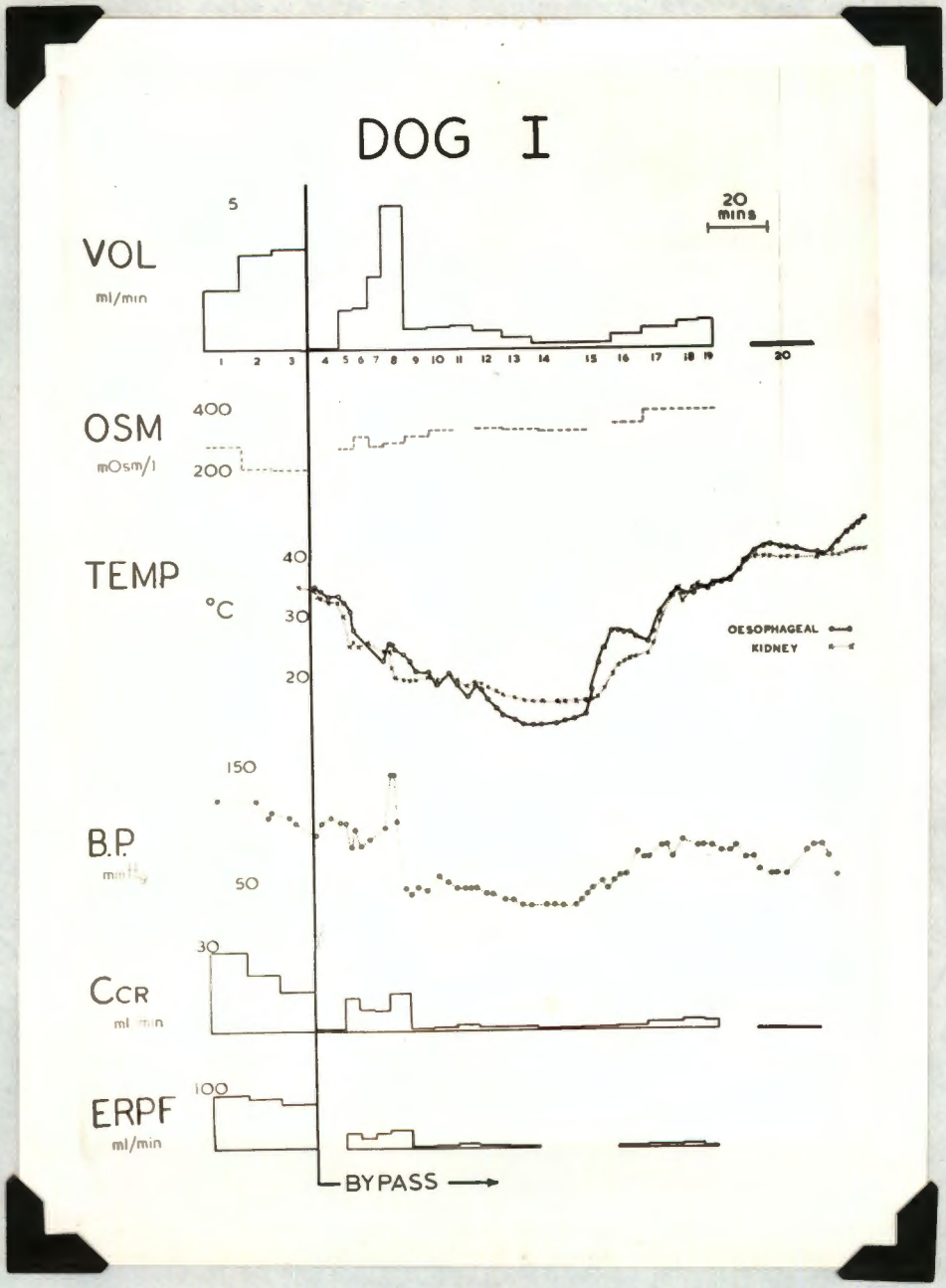

DOG I
VOL
ml/min
5
20
mins
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
OSM
mOsm/l
400
200
TEMP
°C
40
30
20
OESOPHAGEAL
KIDNEY
B.P.
150
50
CCR
ml/min
30
ERPF
ml/min
100
BYPASS

FIG. 23.

made to keep the renal temperature constant. During period 8, active blood stream cooling was resumed. Again, renal temperature fell faster than did the oesophageal, so that at one point it was 4.7° lower. As cooling progressed, however, renal temperature fell little further, while the oesophageal temperature fell progressively. By period 9, the oesophageal temperature was at a low of 11.7°, while the kidney temperature never fell below 15.4°.

Active rewarming brought about a reversal in direction, but not extent, of the trends seen on cooling, in urine flow rate, Ccr and ERPF. Ccr and ERPF remained far below previous levels. The urinary osmolality rose to 394 mOsm/l. The B.P. rose from 30 to 80 mm Hg. The oesophageal temperature rose more rapidly than did the renal temperature; during period 15, it was 7.5° higher than that of the kidney. As the temperature approached normothermic levels (37°), renal and oesophageal temperatures were once again identical.

The attempt at hyperthermia (period 20, and after) raised the oesophageal temperature to a final level of 46°; the renal temperature never rose above 40.7°. Long before this however, the urine flow rate had fallen to zero; during period 20, only 2 ml of urine were collected, and this in the first few minutes only. No

urine was produced thereafter. The blood pressure dropped from 80 mm Hg to a low of 55 mm Hg. Just prior to the conclusion of the experiment, an attempt was made to raise the blood pressure by increasing the perfusion rate; this lead to a transitory return of blood pressure to 80 mm Hg.

THE PERCENTAGE EXCRETION OF FILTERED WATER AND Na:

During the initial 'control' periods 1 to 3, the percentage excretion of filtered water rose from 7.5% to 25.9%, while that of Na rose from 2.2% to 3.7%.

The introduction of bypass caused an immediate drop in the excretion of water, to 14.3% of that filtered. The Na content of the urine collected during this period was not determined.

Active blood stream cooling (commencing with period 5) was accompanied by an irregular increase in percentage excretion, so that by period 14 (mean kidney temperature 15.4°), 58.5% of the filtered Na, and 69.8% of the filtered water, appeared in the urine. During period 9, the systolic arterial blood pressure plunged abruptly from 100 mm Hg to 40 mm Hg; the percentage excretion of water rose, transiently, from 38.6% (period 6) to 87.1%, only to fall to 60.5% in period 10. Na excretion fell from 33.6% (period 8) to 12.6%, then rose to 43.9% in period 10.

On rewarming, less and less of the filtered Na and water appeared in the urine. By period 19 (mean kidney temperature 34.9°) only 0.2% of filtered Na, and 24.4% of filtered water, were excreted.

The mild renal hyperthermia induced in period 20 (average kidney temperature 39.6°) was accompanied by little alteration in percentage excretion of Na (0.5%) and water (29.0%) from that present during normothermia (period 19).

SERUM Na, CREATININE AND OSMOLAR CHANGES:

Serum osmolality rose from 304-306 mOsm/l., to 316-319 mOsm/l., during hypothermia. It then fell to 310 mOsm/l. in blood sample 14, at the start of hyperthermic period 20.

Serum creatinine rose from 4.2 mg/100 ml to 5.5 mg/100 ml only to fall to 3.8 mg/100 ml with the introduction of bypass. After a further slight fall, it then rose steadily and slowly to 6.0 mg/100 ml during urine collection period 16, whereafter the rate of rise increased sharply, so that serum creatinine was 12.9 mg/100 ml at the start of period 20. The last serum sample had a creatinine content of 10.4 mg/100 ml.

Hypothermia was accompanied by a fall in serum Na

from 130 meq/l to a low of 119.5 meq/l (period 9, mean kidney temperature 15.9°), approximately at which level it persisted until the hyperthermic period when it rose to 124.0-126.5 meq/l.

PAH concentration changes paralleled those of creatinine.

COMMENT:

This was the first time we had measured the temperature of the kidney itself. As pointed out in Chapter 11, we had previously felt it desirable to avoid the necessarily traumatic positioning of a thermistor probe in the substance of the kidney, while studying renal function. Now however we were not so much interested in kidney function, as renal temperature, and especially its relationship to that of the oesophagus, during cooling and rewarming. Consequently, no attempt was made here to follow changes in the renal handling of Ca, Mg or inorganic phosphorus.

The most striking result of this experiment was the great discrepancy between renal and oesophageal temperatures, and in particular the varying relationship of the one to the other. The latter was quite unexpected, and was certainly a more than adequate explanation for the variability in apparent activation energies commented upon in Chapter X.

The drop in arterial systolic blood pressure, to 55 mm Hg, during the mildly hyperthermic period is presumably the cause of the anuria experienced here; although if this is so, it is not clear why some urine was not formed when the blood pressure was, temporarily, elevated to 80 mm Hg.

EXPERIMENTAL DATA ON DOG I.

WEIGHT: Not recorded.

INFUSIONS: 1. 10% dextrose water.
2. Creatinine 1.0 G, and PAH 0.65G, in N saline

PRIMED at 12.15 p.m., with creatinine 0.5G., and PAH 0.25 G.

EQUILIBRATION PERIOD: 30 minutes

URINE COLLECTION via polyethyene catheter in left ureter; tip not quite in renal pelvis.

TIMES OF BLOOD AND URINE COLLECTION:

BLOOD SAMPLES:	(1)	(2)	(3)	(4)	(5)		(6)		(7)		(8)		(9)
	12.50	1.01	1.10	1.22	1.33		1.42		1.54		2.10		2.28
Urine collection periods:	12.45 to 12.57 (1)	to 1.08 (2)	to 1.20 (3)	to 1.30 (4)	to 1.35 (5)	to 1.40 (6)	to 1.45 (7)	to 1.52 (8)	to 2.00 (9)	to 2.08 (10)	to 2.15 (11)	to 2.25 (12)	to 2.37 (13)

BLOOD SAMPLES:	(10)	(11)	(12)	(13)		(14)	(15)	
	2.46	2.59	3.11	3.27		3.48	4.08	
Urine collection periods:	to 2.52 (14)	to 3.02 (15)	to 3.12 (16)	to 3.24 (17)	to 3.31 (18)	to 3.36 (19)	3.49 to 4.10 (20)	...

Note: (1) The 10% dextrose water infusion was begun 10 minutes before the first urine collection period started.
(2) A temperature probe was inserted into the substance of the left kidney, immediately after the ureter had been catheterised.
(3) Both drips were slowed at the commencement of bypass, and speeded up again when the renal temperature regained normothermic levels i.e. after period 19.

Cardiopulmonary bypass was begun at 1.20 pm., active blood stream cooling at 1.30 p.m., and active rewarming at 2.52 p.m.

OBSERVED DATA.

URINE

Urine No.	1	2	3	4	5	6	7	8
Volume (ml)	245	35.8	41.0	0.7	6.8	7.0	12.5	34.0
Osmolality (mOsm/L)	275	200	198	-	265	310	271	285
Creatinine (mg/100 ml)	56.6	28.7	21.3	25.7	26.5	16.9	9.6	9.6
Na (meq/l)	37.2	21.1	18.2	-	43.0	59.0	104.0	108.0
≠ PAH (Klett units)	355	250	240	-	106	76	60	42

Urine No.	9	10	11	12	13	14	15	16
Volume (ml)	5.4	5.5	5.5	6.2	4.9	3.5	2.2	4.8
Osmolality (mOsm/l)	306	325	-	330	328	325	-	357
Creatinine (mg/100 ml)	4.4	6.6	10.9	8.0	11.6	6.5	11.6	15.2
Na (meq/l)	18.0	89.8	76.3	85.0	96.0	101.5	101.5	54.0
≠ PAH (Klett units)	39	46	80	57	58	-	-	105

Urine No.	17	18	19	20
Volume (ml)	8.3	5.8	4.3	2.0
Osmolality (m/osm/l)	394	394	395	-
Creatinine (mg/100 ml)	26.8	39.9	41.3	39.1
Na (meq/l)	8.5	2.0	1.0	2.0
≠ PAH (klett units)	142	152	135	-

≠ Urine diluted one hundred times for PAH estimation.
The absolute values of PAH, in mg/100 ml, were not estimated, as these are unnecessary for the simple calculation of ERPF, or C_{PAH}, where both urine and serum concentrations are expressed in the same units.

BLOOD

Blood Sample No.	1	2	3	4	5	6	7	8.	9.
Osmolality (mOsm/l)	-	304	306	305	306	317	-	315	316
Creatinine (mg/100 ml)	4.2	4.7	5.5	3.8	3.4	3.6	3.8	4.1	4.6
Na (meq/l)	130	-	129	129	130	125	123.5	123.5	119.5
≠ PAH (Klett units)	39	46	59	46	32	32	38	38	44

Blood Sample No.	10	11	12	13	14	15
Osmolality (mOsm/l)	316	-	319	316	310	-
Creatinine (mg/100 ml)	4.6	5.4	6.0	8.8	12.9	10.4
Na (meq/l)	120	120.5	-	120.5	124.0	126.5
≠ PAH (Klett units)	46	52	72	107	185	-

≠ Serum diluted twenty times for PAH estimation.

Time	Temperature °C			Systolic Arterial B.P. mmHg
	Oesophagus	Kidney	Rectum	
12.48	-	-	-	120
1.01	-	-	-	120
.05	-	-	-	105
.06	-	-	-	110
.12	-	-	-	105
.14	-	-	-	100
.16	-	35.0	-	-
.21	35.0	34.6	34.4	90
.23	34.2	33.2	34.2	100
.26	33.2	32.6	32.0	105
.29	32.5	32.5	32.7	100
.31	32.2	30.0	31.2	100
.33	30.8	25.0	28.4	80
.34	27.6	25.6	28.2	95
.36	-	25.0	28.0	80
.39	-	25.8	27.6	86
.44	22.5	24.0	26.6	95
.46	25.3	24.0	26.0	140
.47	25.2	21.2	25.0	140
.48	24.5	19.8	24.0	100
.51	23.6	19.4	22.8	45
.53	22.5	19.2	23.0	40
.55	20.8	19.2	22.7	45
.59	20.6	19.8	22.7	50
2.02	18.2	19.1	22.4	43
.06	20.1	20.1	22.4	55

Time	Temperature °C			Systolic Arterial B.P. mmHg.
	Oesophagus	Kidney	Rectum	
2.09	18.3	19.1	22.4	50
.12	16.2	18.1	22.1	45
.15	18.4	19.0	22.0	45
.17	17.7	18.8	22.0	45
.19	16.0	18.0	21.9	45
.22	14.5	17.2	21.6	40
.24	13.5	16.8	21.3	40
.28	12.4	16.2	20.7	35
.31	11.9	15.8	20.3	35
.34	11.7	15.6	19.9	30
.37	11.7	15.5	19.8	30
.42	11.9	15.4	19.2	30
.45	12.2	15.4	19.0	30
.48	12.5	15.4	18.8	30
.52	13.2	15.5	18.7	30
.54	17.4	15.8	18.9	35
.56	22.0	16.2	19.0	40
.58	24.5	17.9	19.3	45
3.01	27.6	20.1	20.0	50
.03	27.2	21.7	20.6	45
.05	27.0	22.1	20.9	50
.07	27.2	22.9	21.3	55
.09	26.2	23.0	21.6	55
.13	25.6	23.5	22.0	75
.15	27.2	25.2	22.2	70
.17	30.6	29.0	22.6	70

Time	Temperature °C			Systolic Arterial B.P.
	Oesophagus	Kidney	Rectum	
3.21	33.2	33.0	23.4	80
.23	34.3	34.5	23.8	80
.25	33.7	32.0	24.2	70
.28	33.4	34.6	24.5	85
.30	34.4	35.1	24.8	-
.33	34.3	34.3	25.1	80
.35	35.0	35.4	25.8	80
.38	35.2	35.4	25.8	80
.41	35.9	36.2	26.1	75
.44	37.2	37.5	26.5	75
.46	39.0	39.0	26.9	80
.49	40.8	39.7	27.1	70
.52	41.4	39.7	27.5	70
.54	41.8	39.6	28.2	60
.58	41.2	39.6	29.6	55
4.00	41.0	39.5	32.6	55
.03	41.0	39.5	32.1	55
.10	40.1	39.4	32.4	75
.12	39.9	39.5	33.5	80
.15	40.4	39.6	34.3	80
.17	42.0	39.6	35.2	70
.20	43.2	40.1	36.3	55
.22	44.1	40.3	36.2	-
.24	45.0	40.4	36.9	-
..26	46.0	40.7	37.0	-

DERIVED DATA.

PERIOD	1	2	3	4	5	6	7	8	9	10
Volume (ml/minute)	2.04	3.25	3.42	0.07	1.36	1.40	2.50	4.86	0.68	0.69
Midperiod Serum:										
Creatinine (mg/100 ml)	4.25	4.83	5.5*	3.69	3.42	3.50	3.61	3.71	3.84	3.99
Na (meq/l)	130.0	129.4	129.0	129.3	130.0	127.5	124.5	124.2	123.5	123.5
ǂ PAH (klett units)	39.6	48.2	54.7	42.2	32.6	32.0	32.2	35.3	38.0	38.0
(Kidney)										
Average Temperature °C	-	-	-	33.1	27.3	25.4	24.5	20.7	19.4	19.5
Cer (ml./min.)	27.1	19.3	13.2	0.49	10.52	6.76	6.65	12.55	0.78	1.14
ERPF (ml./min.)	91.2	84.4	75.1	-	22.1	16.6	23.3	28.9	3.50	4.17
% Excretion of filtered:										
Na	2.2	2.8	3.7	-	4.3	9.6	31.3	33.6	12.6	43.9
H_2O	7.5	16.9	25.9	14.3	12.9	20.7	38.6	38.6	87.1	60.5

PERIOD	11	12	13	14	15	16	17	18	19	20
Volume (ml/minute)	0.79	0.62	0.41	0.23	0.22	0.48	0.69	0.83	0.86	0.09
Midperiod Serum:										
Creatinine (mg/100 ml)	4.14	4.38	4.60	4.60	5.27	5.80	7.22	8.89	10.07	11.46
Na (meq/l)	123.2	121.3	119.6	120.0	120.4	120.5	120.5	120.6	121.6	125.4
ǂ PAH (Klett units)	38.5	41.3	44.3	45.8	51.1	60.0	87.3	108.9	155.3	-
(Kidney)										
Average Temperature °C	18.7	18.2	15.9	15.4	17.9	22.4	29.2	33.8	34.9	39.6
Cer (ml./min.)	2.08	1.13	1.03	0.33	0.49	1.26	2.56	3.73	3.53	0.31
ERPF (ml./min.)	8.32	4.27	2.68	-	-	4.2	5.61	5.81	3.75	-
% Excretion of filtered:										
Na	23.5	38.5	32.1	58.5	38.2	17.1	1.9	0.4	0.2	0.5
H_2O	38.0	54.8	39.8	69.8	45.0	38.1	26.9	22.3	24.4	29.0

ǂ Serum diluted twenty times for PAH estimation. * Actual creatinine concentration of blood 3 is preferable to calculated midperiod value, here.

CHAPTER XII.

DOG J.

The irregular relationship found between renal and oesophageal temperatures in Dog I, had come as an unpleasant surprise. We decided to repeat our observations on oesophageal, renal and rectal temperatures, before during and after the induction of hypothermia, in Dog J.

The consistently low apparent activation energies for the renal reabsorption of inorganic phosphate (cf discussion in a later Chapter) had also intrigued us, and it was accordingly decided to note the effect of Parathormone during hypothermia, as well. Inasmuch as the results of this latter manoeuvre fall beyond the scope of this Thesis, they will not be referred to again. Only the data relevant to previous Chapters will be given here.

The experimental procedure adopted was similar to that followed in Dog I, with two exceptions. Parathormone (200 I.U.) was given 33 minutes before the first urine collection period. Urine was not collected throughout the bypass period, but only intermittently, when the renal temperature was at approximately 15°, and again at 10°.

RESULTS:

The observed relationship between oesophageal renal and rectal temperatures, is presented graphically in Fig. 24. Details of the experimental procedure, and of relevant observed and derived data are given at the end of this Chapter.

OESOPHAGEAL, RENAL AND RECTAL TEMPERATURE RELATIONSHIPS. (FIG. 24)

During the initial 10 minutes of observation, oesophageal renal and rectal temperatures were identical, at 32.0° to 31.4°C.

The introduction of bypass with active blood stream cooling, lead to an immediate and precipitous drop in renal temperature, so that 3 minutes later it was 14° below those of the oesophagus and rectum. The rate of fall in kidney temperature then diminished, and was even transiently reversed, before the kidney reached a low of 9.9°C. Oesophageal and rectal temperatures fell in parallel until reaching 20°, at which point the rate of rectal cooling lagged behind that of the oesophagus. The lowest oesophageal temperature attained was 9.0°, some 4° lessthan that of the oesophagus. Oesophageal and renal temperatures approached and then met each other at 10.2°, 35 minutes after the introduction of bypass and cooling. After this, renal

temperatures ran slightly higher than oesophageal.

Rewarming led to a rapid reversal of these changes. The renal temperature rose, steeply, closely followed by the rise in oesophageal temperature. The rectal temperature however remained low, and even fell slightly, during the first 5 minutes of rewarming. It then rose, but less steeply, than had the renal and oesophageal temperatures.

COMMENT:

In sequence of events, but not in magnitude, the relationship between renal, oesophageal and rectal temperatures during the bypass period were similar in Dogs I and J (cf Fig. 24). They differed in the rewarming period, in so far as the renal response was more rapid than the oesophageal in Dog J, while the converse was true in Dog I. The overall rate of cooling was much faster in Dog J, which may be the reason for the greater discrepancy between renal and oesophageal temperatures at this time. Rectal and oesophageal temperatures diverged at 22.5° in Dog I, as compared to 20° in Dog J.

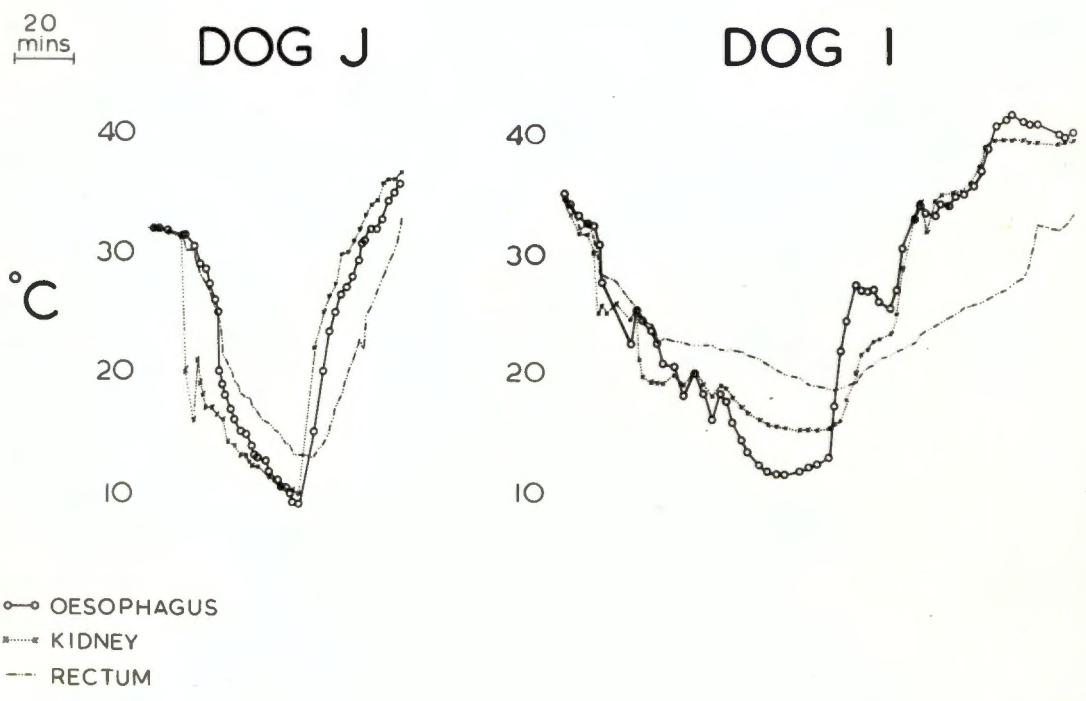


FIG. 24.

EXPERIMENTAL DATA ON DOG J.

WEIGHT: 21.0 Kg

INFUSIONS: (1) 10% dextrose water
(2) Creatinine 1.0g in 5% dextrose water.

PRIMED at 11.30 a.m., with Creatinine 0.5G.

EQUILIBRATION PERIOD: 40 minutes

URINE COLLECTION via polyethylene catheter in left ureter; tip in renal pelvis.

TIMES OF BLOOD AND URINE COLLECTIONS:

BLOOD SAMPLES: ① 12.1$4\frac{1}{2}$ ② 12.3$5\frac{1}{2}$ ③ 12.49 ④ 12.59

Urine collection periods:

12.10 to 12.15 (1)	to 12.20 (2)	12.30½ to 12.3½ (3)	to 12.40½ (4)	12.50 to 12.55 (5)	to 1.00 (6)

Cardiopulmonary bypass begun, with simultaneous active blood stream cooling at 12.20 p.m.; kidney temperature held at approximately 16° during periods 3 and 4, and at approximately 10° during periods 5 and 6.

The infusion of 10% dextrose water was discontinued at 12.25 p.m., five minutes after bypass was commenced.

Note: (1) Parathomone (200 I.U.) was given i.v. at 11.37 a.m., 33 minutes before urine collection No. 1 was begun.
(2) A temperature probe was inserted into the substance of the kidney immediately after the ipsilateral ureter had been catheterised.

OBSERVED DATA.

URINES

Urine No.	1	2	3	4	5	6
Volume (ml)	11.5	9.6	14.0	13.9	10.9	11.6
Creatinine (mg/100 ml)	24.0	21.1	7.68	6.02	5.76	5.76

BLOOD

Blood Sample No.	1	2	3	4
Creatinine (mg/100 ml)	4.7	3.9	4.6	4.8

DERIVED DATA.

PERIOD	1	2	3	4	5	6
Volume (ml/min)	2.30	1.92	2.80	2.78	2.18	2.32
Midperiod Serum:						
creatinine (mg/100 ml)	4.70	4.59	4.00	4.03	4.67	4.77
Average Kidney Temperature °C	31.8	31.5	16.1	13.7	10.7	10.0
Cer (ml/min)	11.8	8.8	5.4	4.1	2.8	2.9

DOG J.

Time	Temperature °C Oesophageal	Renal	Rectal	Arterial Systolic B.P. (mmHg)
12.10	32.0	32.0	32.0	-
.12	32.0	31.8	31.8	98
.15	31.8	31.6	31.6	80
.20	31.4	31.4	31.4	85
.21	31.4	20.0	30.0	55
.24	30.4	16.0	30.0	45
.25	30.0	21.0	29.0	110
.26	29.0	19.0	28.0	-
.27	28.8	18.0	27.4	70
.28	27.6	17.0	26.8	-
.29	27.4	17.0	26.0	-
.30	27.0	17.0	25.2	70
.31	26.0	-	24.4	-
.32	25.0	16.4	24.0	-
.33	20.0	16.1	21.4	65
.34	19.0	16.0	21.0	-
.35	18.1	-	20.4	-
.36	17.0	14.2	20.0	-
.37	16.8	14.0	19.2	55
.38	16.0	13.8	18.8	-
.39	15.0	13.4	18.2	-
.40	15.0	13.0	18.0	-
.41	14.8	13.0	17.8	-
.42	14.8	13.0	17.8	-
.43	14.0	12.4	17.0	-
.44	13.8	12.2	16.8	

DOG J.

Time	Temperature °C			Arterial Systolic B.P. (mmHg)
	Oesophageal	Renal	Rectal	
12.45	13.0	12.0	16.4	48
.46	12.8	12.0	16.0	
.49	12.6	11.6	15.6	
.50	11.7	11.2	15.2	
.52	11.2	11.0	14.8	
.53	11.0	10.4	14.6	
.54	10.4	10.2	14.0	
.55	10.2	10.2	14.0	
.56	10.4	10.1	13.6	
.57	9.8	10.0	13.2	
.58	9.2	10.0	13.0	
1.00	9.0	9.9	13.0	
.05	15.0	22.0	12.9	
.08	20.0	25.0	14.0	
.10	23.4	26.4	15.0	
.12	25.0	27.4	17.0	
.14	26.4	29.9	18.0	
.16	27.2	30.0	19.9	85
.18	28.0	31.0	21.2	
.20	29.4	32.0	22.9	75
.21	30.8	33.0	22.0	
.22	31.0	33.1	24.6	65
.24	32.0	34.0	25.4	
.26	32.0	34.4	27.0	60
.28	32.8	35.8	28.2	63
.30	34.4	36.2	29.4	
.32	35.0	36.2	30.4	
.34	35.0	36.8	31.8	
.36	35.8	36.8	32.8	

CHAPTER XIII

DOGS K and L.

Direct measurement of renal and oesophageal temperatures during the induction of, and recovery from, hypothermia had shown (Dogs I and J) that renal and oesophageal temperatures were not necessarily identical, particularly during periods of rapid alterations in temperature. Even worse was the variable relationship of the one to the other, which makes the calculation of apparent activation energies of renal processes, when equating oesophageal with kidney temperatures, quite unreliable.

To obtain an accurate estimate of the activation energy of, for example, the renal reabsorption of Na, it seemed essential then to measure the renal temperature itself. Furthermore, temperature gradients can conceivably exist even within the kidney; this would make it desirable to hold the kidney temperature constant for a period before collecting urine, in an attempt to allow these gradients time in which to dissipate themselves.

The aim of the two experiments recorded in this Chapter was simply to ascertain if the data obtained under the above conditions would yield comparable values for the apparent activation energy of the renal reabsorption of Na.

The experimental procedure adopted was similar to that for Dog J, with the following exceptions: Urine and blood samples were collected only after bypass and hypothermia had been initiated, and then at only two (constant) kidney temperatures, viz. 25° and 6° in Dog K, and at 25° and 12° in Dog L. No attempt was made to study renal function during rewarming. Neither parathormone, nor other extraneous substances, were administered during these experiments.

RESULTS:

The comparisons between renal and oesophageal temperatures, for both dogs K and L, are presented graphically in Fig. 25. Details of experimental, observed and derived data, for both dogs, are given at the end of this Chapter.

COMPARISON OF OESOPHAGEAL AND RENAL TEMPERATURES DURING CARDIOPULMONARY BYPASS AND HYPOTHERMIA, IN DOGS K AND L. (Fig. 25)

Both dogs show a more rapid and a greater fall in renal than oesophageal temperature on initiation of bypass with active blood stream cooling. On then holding the renal temperature constant at close to 25°, renal and oesophageal temperatures soon became identical. The kidney temperature was held at approximately 25° for 46 minutes in Dog K, during the last 30 minutes of which urine was collected. In Dog L,

the kidney was at about 25° for 60 minutes, and urine was collected during the last 30 minutes of this time.

On then cooling to the lowest temperature possible under the particular circumstances of the individual experiments, renal temperature fell to a low of 5.9° in Dog K, and 11.9° in Dog L. In each case, renal temperature fell more rapidly than did the oesophageal. In Dog K, oesophageal temperature ultimately fell to about 0.5° less than the renal temperature, while in Dog L oesophageal temperature never reached that of the kidney, but remained 0.5° above it.

The renal temperature of Dog K was kept at about 6°C, for 44 minutes, during the last 35 minutes of which urine was collected. In Dog L, the renal temperature was held at approximately 12°C for 70 minutes, and urine collected during the last 35 minutes of this period.

PERCENTAGE EXCRETION OF FILTERED Na:

In Dog K, the percentage excretion of filtered Na, with a renal temperature of 25°, was fairly constant, at 12.3% to 10.1%. At 6°, the percentage excretion rose from 54.5% during urine collection period 4, to 66.7% in period 5.

Dog L showed a much greater variation in percentage excretion of filtered Na at constant renal temperatures.

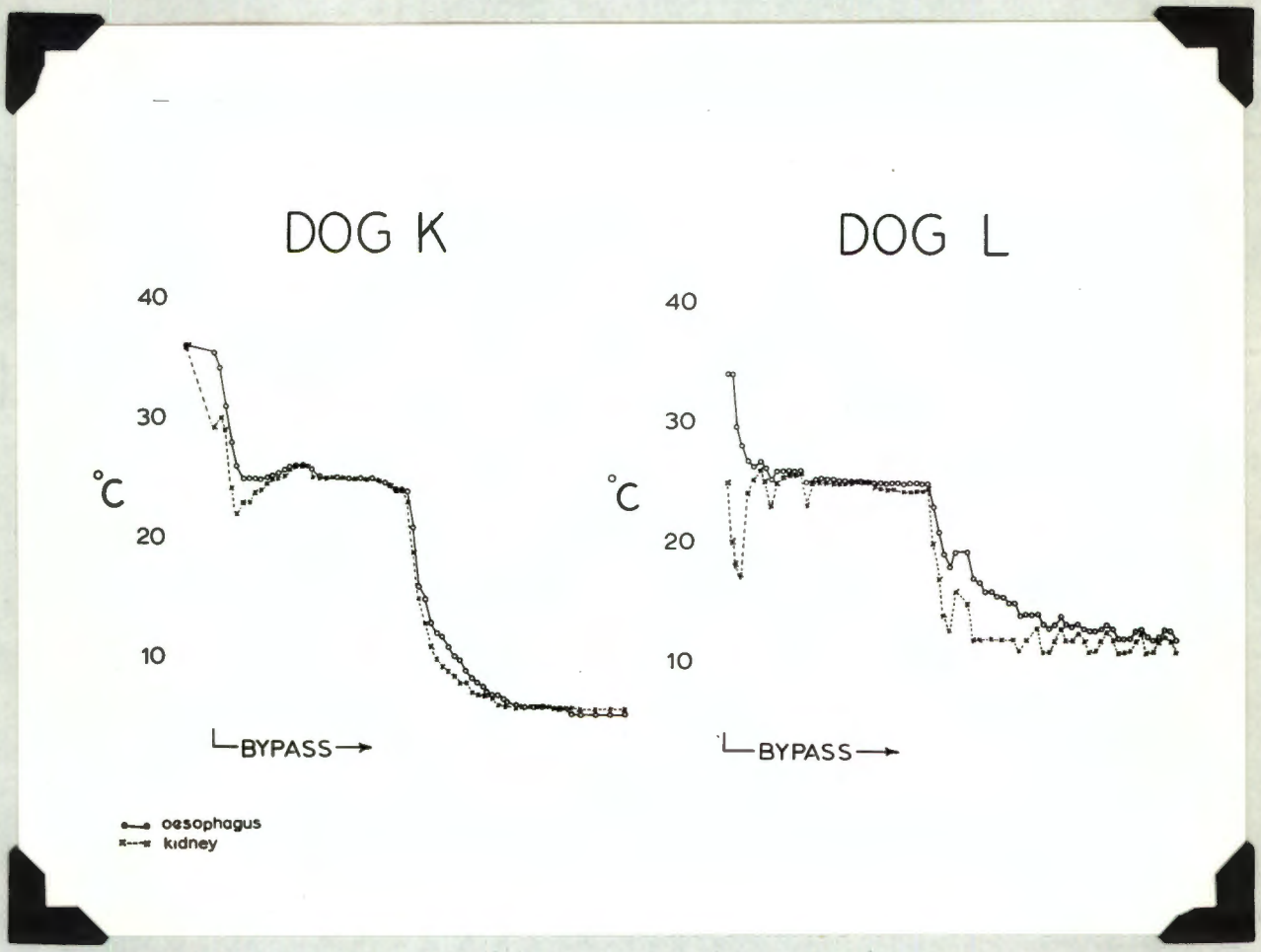


FIG. 25.

Thus, when at 25°, renal excretion of Na fell from 25.5% to 10.4%. At 12°, it rose from 26.2 to 39.8%

THE CALCULATED APPARENT ACTIVATION ENERGY OF RENAL REABSORPTION OF Na.

The figures for the 'corrected' reabsorption of Na are needed at only two temperatures to apply the equation (cf Chapter X and Appendix E).

$$\log\frac{(K1)}{(k2)} = E\,\frac{(T1-T2)}{2.3R\,(T_1 . T_2)}$$

DOG K. K_1 and T_1 are derived from the means of the figures for percentage Na excretion in periods 1 to 3, and 25°C, respectively. As urine collection period 4 was begun after only 9 minutes at 6°, and as the percentage excretion of Na here differs markedly from that of urine collection period 5, k_2 is derived from the percentage excretion in period 5 only. T_2 is derived from 6°C.

Now:

$$\log\frac{(k1)}{(k_2)} = \frac{E(T_1 - T_2)}{2.3R\,(T1T2)}$$

$$\text{i.e. } \log\frac{(88.8)}{(32.3)} = \frac{.214\ E\ (19)}{298.3 \times 279.3}$$

$$\text{i.e. } E = \frac{.438 \times 83,000}{.214 \times 19}$$

$$= 8950 \text{ cals./degree}$$

DOG L: The perfusion rate during urine collection periods 1 to 3 changed from 1500, to 800 and then to 1300 ml/minute.

This was accompanied by large changes in Ccr and calculated percentage excretion of filtered Na. One may expect the percentage excretion to fall with a fall in Ccr (cf discussion in a later Chapter), and so perhaps explain the drop in percentage excretion between periods 1 and 2, from 25.5% to 16.4%. During urine collection period 3, however, the Ccr rises (slightly), yet the percentage excretion of filtered Na drops further. This makes it difficult to explain away the lesser percentage excretion of Na in period 3, and impossible to guess which, if any, of these periods represent the 'real' value of percentage excretion for this temperature. No doubt the large changes in perfusion rate during these urine collection periods are responsible for the large changes in percentage excretion. If one assumes, arbitrarily, that period 3 is the most 'correct', then k_1 must be derived from 10.4%, and T_1 from 24.2°C.

Urine collection period 4 was collected after 35 minutes of a constant renal temperature of 12°C. Yet the percentage excretion of filtered Na differs markedly in periods 4 and 5. The mean of these figures is 33% excretion of Na, at 12°C.

Now the calculated apparent activation energy for the renal reabsorption of Na is:

$$E = \frac{\log\frac{(89.6)}{(67.0)} \times 297.2 \times 285}{.214 \times 12.2}$$

$$= \frac{.112 \times 84,800}{.214 \times 12.2}$$

$$= 3630 \text{ cals./degree.}$$

It is obvious that the great discrepancies found in percentage excretion at near-identical temperatures, in the various urine collection periods, render the above calculation almost meaningless.

EXPERIMENTAL DATA ON DOG K.

WEIGHT: 23.2 Kg

INFUSIONS: (1) 10% dextrose-water
(2) Creatinine 1.5G in 5% Dextrose-N saline.

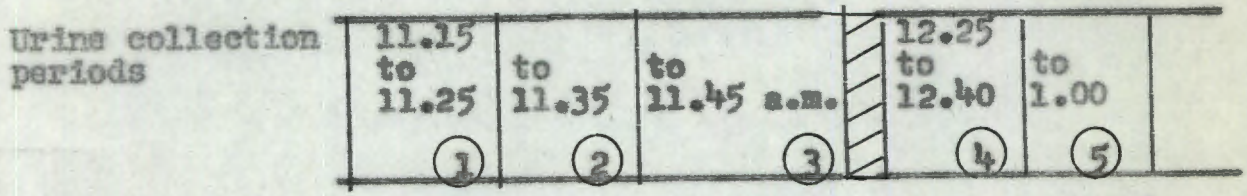
PRIMED at 10.0 a.m. with 0.5G creatinine

EQUILIBRATION PERIOD: 75 minutes

URINE COLLECTION via polyethylene catheter in left ureter; tip in renal pelvis.

TIMES OF BLOOD AND URINE COLLECTIONS:

BLOOD SAMPLES: ① 11.15 ② 11.27 ③ 11.42 ④ 12.22 ⑤ 12.39 ⑥ 12.55



Cardiopulmonary bypass begun at 10.40 a.m, with simultaneous active blood stream cooling. Temperature then held constant at 25° during urine collection periods 1 to 3, and at about 6° during urine collection periods 4 and 5.

NOTE: (1) ALL urines blood tinged.

(2) A temperature probe was inserted into the substance of the kidney, immediately after the ipsilateral ureter had been catheterised.

DOG K.

Time	Temperature °C Oesophageal	Kidney	Time	Temperature °C Oesophageal	Kidney
10.30	36.0	36.0	11.32	25.0	24.9
.40	35.4	29.1	.34	25.0	24.9
.42	34.2	30.0	.36	24.9	24.9
.44	31.0	29.0	.38	24.8	24.6
.46	28.0	24.2	.40	24.6	24.4
.48	26.0	22.0	.42	24.2	24.1
.50	25.0	23.0	.46	24.0	23.1
52	25.0	23.0	.48	21.0	19.0
54	25.0	23.8	.50	16.0	15.0
56	25.0	24.0	.52	15.0	13.0
58	25.1	24.6	.54	13.0	11.1
11.00	25.3	25.0	.56	12.2	10.0
.02	25.5	25.0	.58	11.9	9.4
.04	25.8	25.1	12.00	11.0	9.0
.06	26.0	25.8	.02	10.1	8.6
.08	26.0	26.0	.04	9.9	8.0
.10	26.0	26.0	.06	9.0	8.0
.12	26.0	26.0	.08	8.4	7.2
.14	25.8	25.1	.10	8.0	7.0
.16	25.1	25.0	.12	7.6	7.0
.18	25.1	25.0	.15	7.0	6.8
.20	25.1	25.0	.16	7.0	6.4
.22	25.1	25.0	.18	6.8	6.2
.24	25.1	25.0	.20	6.4	6.1
.26	25.0	25.0	.23	6.2	6.0
.28	25.8	25.0	.25	6.2	6.0
.30	25.0	25.0			

DOG K.

Time	Temperature °c Oesophageal	Kidney
12.28	6.0	6.0
.30	6.0	6.0
.32	6.0	6.0
.34	6.0	6.0
.36	5.9	6.0
.40	5.8	6.0
.42	5.4	6.0
.45	5.4	5.9
.50	5.4	5.9
.55	5.4	5.9
1.00	5.4	5.9

OBSERVED DATA.

DOG K

URINE

Urine No.	1	2	3	4	5
Volume (ml)	11.6	11.6	9.6	4.1	2.3
Creatinine (mg/100 ml)	21.9	25.5	28.2	6.75	5.63
Na (meq/l)	142	142	142	130	130

BLOOD

Blood Sample:	1	2	3	4	5	6
Creatinine (mg/100 ml)	2.7	2.9	3.0	3.5	3.7	3.8
Na (meq/l)	147	147	149	134	126	130

DERIVED DATA.

PERIOD	1	2	3	4	5
Urine volume (ml/min)	1.16	1.16	0.96	0.27	0.12
Midperiod serum:					
Creatinine (mg/100 ml)	2.79	2.92	2.99	3.62	3.87
Na (meq/l)	147	147.5	148.8	129.0	128.8
Average Temperature °C (Renal)	25.0	25.0	24.5	6.0	5.9
Ccr (ml/min)	9.1	10.1	9.1	0.50	0.18
Percentage excretion of filtered:					
Na	12.3	11.1	10.1	54.5	66.7
H_2O	12.8	11.5	10.5	54.0	66.9

EXPERIMENTAL DATA ON DOG L.

INFUSION: Creatinine 1.5 G in 10% Dextrose-water

PRIMED at 10.02 a.m. with 0.5 G creatinine

EQUILIBRATION PERIOD: 55 minutes

URINE COLLECTION via polyethylene catheter in left ureter; catheter tip in renal pelvis.

TIMES OF BLOOD AND URINE COLLECTIONS:

BLOOD SAMPLES:

(1)	(2)	(3)	(4)	(5)	(6)	(7)
10.56	11.06	11.20	11.28	12.01	12.21	12.44

Urine collection periods:

10.57 to 11.07 (1)	to 11.7 (2)	to 11.27 (3)		12.02 to 12.22 (4)	to 12.52 (5)

Cardiopulmonary bypass and active blood stream cooling begun at 10.19 a.m.

Infusion stopped just prior to beginning urine collection one. The kidney temperature was held at 25° during urine collection periods 1 to 3, and at about 12° during urine collection periods 4 and 5.

NOTE: (1) Perfusion rate changed from 1500 to 800 ml/min at 11.06 a.m., raised again to 1300 ml/min at 11.17 a.m.

(2) A temperature probe was inserted into the substance of the kidney immediately after the ipsilateral ureter had been catheterised.

DOG L.

Time	Temperature °C Oesophageal	Renal
10.19	34.0	25.0
.21	34.0	20.0
.22	29.6	18.0
.24	28.0	17.0
.26	26.8	24.0
.28	26.4	25.2
.30	26.8	26.0
.32	26.2	25.0
.34	25.2	23.0
.36	26.0	25.0
.38	26.0	25.4
.40	26.0	25.6
.42	26.0	25.6
.44	26.0	25.8
.46	25.0	23.0
.48	25.4	25.0
.50	25.4	25.0
.52	25.4	25.0
.54	25.4	25.0
.56	25.2	25.0
.58	25.2	25.0
11.00	25 .1	25.0
.02	25.1	25.0
.04	25.1	25.0
.06	25.1	25.0
.08	25.0	25.0

Time	Temperature °C Oesophageal	Renal
11.10	25.0	24.8
112	25.0	24.6
.14	25.0	24.6
.16	25.0	24.3
.18	25.0	24.3
.20	25.0	24.3
.22	25.0	24.4
.24	25.0	24.4
.26	25.0	24.5
.28	23.0	20.0
.30	21.0	17.0
.32	19.1	14.0
.34	18.0	12.8
.36	19.4	16.0
.40	19.4	15.0
.42	17.0	12.0
.44	16.8	12.0
.46	16.0	12.0
.48	16.0	12.0
.50	15.6	12.0
.52	15.6	12.0
.54	15.0	12.0
.56	15.0	12.0
.58	14.0	11.0
12.00	14.1	12.0

DOG L.

Time	Temperature °C	
	Oesophageal	Renal
12.02	14.1	12.0
.04	14.3	13.0
.06	13.3	11.0
.08	13.0	11.0
.10	13.3	12.0
.12	14.0	13.0
.14	13.3	12.0
.16	13.2	12.0
.18	13.4	12.6
.20	13.0	12.0
.22	12.8	11.0
.24	12.8	11.2
.26	13.0	12.0
.28	13.3	12.8
.30	13.0	12.0
.32	12.2	11.0
.34	12.2	11.0
.36	12.2	11.2
.38	12.9	12.0
.40	13.0	12.8
.42	12.4	11.0
.44	12.4	11.0
.44	12.0	11.0
.46	12.4	12.0
.48	13.0	12.4
.50	12.9	12.0
.52	12.0	11.0

OBSERVED DATA.

DOG L.

URINES

Urine No.	1	2	3	4	5
Volume (ml)	25.5	5.4	4.2	4.0	7.0
Creatinine (mg/100 ml)	17.7	27.0	42.7	17.5	11.3
Na (meq/l)	123	124	130	144	143

BLOODS

Blood Sample No.	1	2	3	4	5	6	7
Creatinine (mg/100 ml)	5.2	5.2	4.9	4.7	4.5	4.5	4.5
Na (meq/l)	142	142	142	141	141	141	143

DERIVED DATA.

Period:	1	2	3	4	5
Urine Volume (ml/min.)	2.55	0.54	0.20	0.42	0.23
Midperiod Serum:					
Creatinine (mg/100 ml)	5.20	5.07	4.85	4.50	4.50
Na (meq/l)	142	142	141.8	141.0	142.4
Average Kidney Temperature °C	25.0	24.7	24.2	12.0	11.9
Creatinine Clearance (ml/min.)	8.66	2.88	3.70	0.78	0.58
% excretion of filtered:					
Na	25.5	16.4	10.4	26.2	39.8
Water	29.5	18.8	11.4	25.6	39.7

CHAPTER XIV

THE RENAL EFFECTS OF EXTRACORPOREAL CARDIO-PULMONARY BYPASS.

In all our experiments, the renal effects of extracorporeal cardiopulmonary bypass underlay those of hypothermia. An appreciation of the former is therefore an essential preliminary to the understanding and interpretation of the latter.

A number of studies on renal circulatory response to cardiopulmonary bypass have recently appeared in the literature. The results may be summed up by the statement that (136, 85) "The renal blood flow during extracorporeal circulation is a function of the perfusion rate, and there is nothing inherent in extracorporeal circulation that precludes relatively normal renal haemodynamics".

Working with dogs, Replogle and Gross (136) found that cardiopulmonary bypass led to a rapid decline in renal blood flow, which continued to fall until it reached 10% to 15% of control values, within about 20 minutes of initiation of bypass. On resumption of normal circulation, the renal blood flow regained control values within 30 minutes. It is important to note that these workers used a perfusion rate of 1800 to 2000 ml/m^2/minute, - or, using Homck's conversion factor (cf Appendix C), 92 to 101ml/kg body weight/minute. To avoid a fall in renal blood flow, the perfusion rate needed to be of the order of 3 litres/m^2/minute, or approximately 152 ml/kg body weight/minute.

Senning et al (151) studied alterations in GFR and electrolyte excretion, as well as of renal plasma flow, during extracorporeal perfusion. They perfused dogs at 100 ml/kg/minute, as well as at 40 ml/kg/min. Bypass was found to lead to a fall in GFR and electrolyte excretion, as well as in renal plasma flow. These effects were most marked at the lower perfusion rate.

These investigations have been supplemented by renal arteriography during extracorporeal circulation. Finsterbusch et al (54) performed renal arteriograms at half hourly intervals on dogs subjected to extracorporeal circulation. They found that immediately bypass began, a marked narrowing and straightening of the main renal arteries occurred, and that the renal cortex showed up poorly. These changes persisted for up to 2 hours.

An isolated observation, applicable to the discussion that follows, is that of Bucknam and Galindo (30). These workers, using extracorporeal circulation in dogs, at flow rates of 40 ml/kg/minute, noticed that even in the absence of active cooling, oesophageal temperature frequently dropped to about 32°C, and ascribed this to cooling of the blood by room air, during its passage through the extracorporeal circuit.

Additional light has been thrown on the changes produced in renal blood flow and urinary composition by cardiopulmonary bypass, by a number of experimenters not directly concerned with this aspect of renal function.

Corday and Williams (39) found that haemorrhage, or shock, led to an immediate and marked fall in renal blood flow, with a rise in renal vascular resistance, and that although this fall was 'disproportionately large', it was readily reversible by blood transfusion. They noticed too, that the renal blood flow could fall even further, despite a subsequent slight rise in blood pressure, a finding which confirmed an earlier observation by Selkurt (148). Their conclusion was that "because of its copious blood supply, and its well known labile vasomotor activity, the kidneys play an important role in the homeostasis of the circulatory system". The validity of this conclusion is perhaps confirmed by an observation of Semb et al (136) who, measuring the renal blood flow directly, found that at normal temperatures the renal blood flow varies considerably within relatively short time intervals.

Lowering the GFR in the normal kidney leads to a fall in percentage excretion of both filtered water and solute (176, 15, 175), and sometimes to a rise of urinary osmolality. Provided that some Na remains in the urine,

and that the fall in GFR is not gross the excretion of K however, is unaffected, and this finding has been interpreted (14) as evidence for the secretion of K, with almost complete reabsorption of such K as may have been filtered.

It has been said that "mathematical reasoning shows that in a counter-current multiplier system, Uosm/Posm is inversely related to the volume of fluid entering the system per unit time" (182). Thus, even in the Hydropenic dog, lowering the GFR leads to a rise in urine osmolality (103). Where the fall in GFR is more than 30% of the normal however, Uosm may decrease. This has been attributed to a greater fraction of the filtered Na being then reabsorbed in the proximal tubule, leaving less available for transport by Henle's loops, and so leads to a lowered medullary interstitial Na concentration, and hence a lowered Uosm.

Inducing a mild diuresis, will now lead to a rise in Uosm, despite the great reduction of GFR, and this presumably is secondary to the now smaller fraction of filtered Na reabsorbed in the proximal tubules. In evaluating the relevance of these experimental findings to those featured in this Thesis, it must be borne in mind that hypertonic urine can be formed in the absence of pituitary ADH (15).

Our dogs had been exposed to normothermic cardio-pulmonary bypass in a number of instances, and in all cases, at a perfusion rate of approximately 70 ml/kg/minute.

In Dog B, and Normothermic Dog 1, normothermic perfusion had followed on normothermic, normo-circulatory control periods, and the results obtained are in precise accordance with the experimental findings of others, as detailed above. Thus urine flow rate and creatinine clearance fell, urinary osmolality rose, and the percentage excretion of filtered electrolytes fell steeply. The excretion of K remained unchanged (Dog B) or fell to a much lesser extent than did the other electrolytes (Normothermic Dog 1).

Normothermic perfusion was also performed during the rewarming of previously hypothermic dogs (Dogs A, B, C, D, E, G and I). A striking finding here was the occurrence of proteinuria, in 5 of the 6 dogs, in which this was sought for (Fig. 26). This will be discussed in the next chapter. For the rest, the effects of normothermic bypass, after preliminary cooling, did not seem to differ significantly from those described above.

It is of interest to note the degree of hypothermia produced by the extracorporeal circulation alone, without active blood stream cooling (Table 1). The oesophageal temperatures of our dogs reached lower levels than the 32°

reported by Bucknam and Galindo (30), a fact no doubt due to the higher perfusion rate in our series (70 ml/kg/min. as compared to 40 ml/kg/min), as well as to the great drop in body temperature which occurred during the period of preparative surgery.

In summary, extracorporeal cardiopulmonary bypass, at perfusion rates of the order used in this study, may be expected to cause a marked fall in the renal blood flow, presumably due to intrarenal vasomotor activity. This produces a drop in the glomerular filtration rate, which in turn leads to a decreased percentage excretion of filtered water and solutes (but not potassium), and rise in urinary osmolality. These changes occur also, immediately after a period of profound hypothermia. The extracorporeal circulation by exposing the circulatory blood to room air, may cause a drop of several degrees in body temperature.

TABLE 1.

The degree of hypothermia produced by the extracorporeal circulation alone, without active blood stream cooling.

DOG	BYPASS without active cooling.			
	Oesophageal Temperature (°C) Initial	Final		Duration (minutes)
C	±32.0	±28.0		10
D	29.4	28.0		9
E	±32.0	27.3		11
G	±37.0	30.3		15
I	35.0	32.5		10
"Normothermic 2"	32.8	30.9		34

CHAPTER XV.

SERUM ELECTROLYTE AND OSMOLAR CONCENTRATION CHANGES DURING PROFOUND HYPOTHERMIA.

Table 2 summarises the changes seen in serum osmolality and electrolyte concentfations during extracorporeal cardiopulmonary bypass. The data have been assembled into two groups: those pertaining to normothermic bypass, and those seen during profound hypothermia.

Dogs B, C, D, E, G, I and J were perfused at 37° for short periods during the rewarming process. The serum electrolyte changes pertaining to these periods have not been included in the normothermic bypass data in Table 2, as they followed immediately upon periods of profound hypothermia and so might well be atypical. The normothermic bypass data shown for Dog B apply to the pre-cooling period; it will be recalled that this was the only dog to be perfused at 37° prior to the induction of hypothermia. "Normothermic" dog 2 is included, as the drop in oesophageal temperature that occurred here was of but a few degrees, and of an entirely different order of magnitude to that produced in the profoundly hypothermic dogs.

Scrutiny of Table 2 reveals that the serum Na, inorganic phosphate and osmolar concentrations underwent changes

similar in direction, during both normothermic and hypothermic bypass. Serum osmolality rose and serum Na and inorganic phosphate concentrations fell, consistently. The changes noted for the other electrolytes were variable, with no apparent differences between normothermic and hypothermic serum concentration trends.

The fall in serum Na concentration, where present, was much greater during profound hypothermia than during normothermic bypass. Thus in Dogs C, D, E, G, I and K, the serum Na concentration fell by 11-20 meq/l (mean, 15 meq/l), whereas, it fell only 3 to 5 meq/l in the normothermic bypass group.

Normothermic extracorporeal cardiopulmonary bypass produces no significant alteration in serum electrolyte concentrations (33), provided that the hypokalaemia of hyperventilation is avoided (45).

A survey of the literature (summarised in Table 3) pertaining to serum electrolyte, glucose, osmolar and protein concentrations during hypothermia reveals complete lack of uniformity of reported findings. These experimenters employed a variety of animals, and with few exceptions (123, 133, 163, 179), did not reduce body temperature to the low levels found in this study. Only one investigator (124) utilised extracorporeal blood stream cooling, and this with an unassisted circulation.

TABLE 2.

Serum Electrolyte and Osmolar Concentration changes during Normo- and Hypo-Thermic Extracorporeal Cardiopulmonary Bypass.

Normothermic Bypass:

Serum Concentration of	DOG B at 37°	Normothermic Dog 1	'Normothermic' Dog 2
Na	↓	∝	↓
K	↓	∝	↔
Ca		↓	↓
Mg	↔	↔	↓
P		↓	↓
Osmolality	↑	↑	↑

Hypothermic Bypass:

Serum Concentration of	Dog B	C	D	E	G	I	J	K	L
Na	↔	↓	↓	↓	↓	↓	↓	↓	↔
K	↓	∝	↓	∝	∝				
Ca	↓	↓	↑		↑				
Mg	↓	∝	∝		∝				
P	↓	↓	↔		↓				
Osmolality	↑	↑	↔		↑	↑			

Meaning of Symbols: ↑ = serum concentration RISES

↓ = serum concentration FALLS

∝ = serum concentration Fluctuates Irregularly.

↔ = serum concentration CONSTANT.

Several factors may be responsible for these discrepancies in reported serum electrolyte concentrations. Wynn (184) has pointed out that as glucose transport across cell membranes is an active process, it may be expected to be inhibited by hypothermia; this would lead to hyperglycaemia, a rise in ECF osmolality, and so an increase in ECF volume, as water is drawn out of cells. This in turn, would produce a fall in serum electrolyte concentrations. He reports a case (a human female) in which the blood sugar, initially 145 mg/100 ml, rose to 1040 mg/100 ml., while the serum Na concentration fell from 135 meq/l to 123 meq/l., during hypothermia. Hyperventilation - i.e. too enthusiastic artificial respiration during anaesthesia - can produce a fall in serum Na, K and inorganic phosphate concentrations, during both hypothermia and normothermia (80, 124, 166). Anaesthesia alone has been said to lead to a fall in serum K concentration (37), while anoxia and acidosis produce a rise in serum K and a fall in serum Na concentration (37, 124). Finally, the extracorporeal circulation produces appreciable haemolysis, and so may be expected to cause a rise in serum K, Mg, and inorganic phosphate concentrations.

Probably all these factors, at one time or another, were in play during our experiments. The most obvious is hyperglycaemia. All our dogs were infused with 10% dextrose water. As pointed out in Chapter 11, this can be expected

TABLE 3

SERUM CONCENTRATION CHANGES DURING HYPOTHERMIA.

Source	Cooling Technique	Na	K	Ca	Mg	P	Glucose	mOsm/l	Protein	Species
Bigelow (20)	Refrigeration Blankets		↑	α						Dog
Fleming (57)	" "	↓	↑	↑	↔	↔				"
Hong (80)	Ice Water Immersion							↔		"
Juvenelle (86)	" " "	α	α	α		↑	↑		↓	"
Lewis (105)	" " "		α							"
Mavor (108)	" " "		↓				↓			"
McMillan (110)	" " "	↔	α	↑	↔					"
Moussa (118)	" " "				↔					"
Moyer (120)	" " "	↔	α							"
Niazi (123	Refrigeration Blankets		α							"
Platner (133)					↑					"
Segar (146)	Ice water Immersion	↔	↓							"
Segar (147)	" " "					↓	↑		↔	"
Swan (166)	" " "	↔	↓			↓				"
Wilder (179)	" " "	↔	↑					↑		"
Woodruff (183)	" " "						↓			"

P.T.O.

TABLE 3. (Cont.)

Source	Cooling Technique	Na	K	Ca	Mg	P	Glucose	Osmolality	Protein	Specie
Boba (24)	Surface Cooling				↑					Man
Laufman (101)	Accidental Exposure	↑	↔				↑		↑	"
Morales (116)	Surface Cooling	↓								"
Moyer (119)	" "	↔	∝							"
Barbour (8)	Cold Air								↑	Monkey rats
Beaton (12)		↔	↔	↔		↑				Rats
Elliot (52)			↑	↑					↑	Rats
Nielsen (124)	Blood stream Cooling		↓	↔					↔	Rabbit
Platner (133)	Surface cooling				↑					Turtle
Platner (132)	" "				↑					Turtle Gold-fish
Smith (156)	Ice water Immersion					↑	↑			
Steadman (163)	" " immersion				↑					Rabbit

Meaning of symbols: ↑ = serum concentration RISES on cooling

↓ = " " FALLS " "

∝ = " " VARIES during cooling.

↔ = " " Remains unchanged during cooling.

to lead to a rise in serum osmolality, as well as a fall in serum Na and inorganic phosphate concentrations, even in normothermic dogs. Following Wynn's postulates, these effects should be exaggerated during hypothermia; comparison of our normothermic and hypothermic bypass data suggests that this was indeed so, particularly as regards the fall in serum Na concentration.

For the rest, hypothermia does not seem to have produced any particular alteration in serum electrolyte concentrations in our experiments. With dilution of the extracellular fluid volume, serum protein and so total serum Ca and Mg concentrations might be expected to fall. As evident in Table 2, these electrolytes were estimated in only four hypothermic dogs, with variable results. Of some interest is the lack of any particular change in serum Mg concentration; it has been known for many years that serum Mg concentration increases considerably during hibernation (105, 120, 156), as well as in goldfish and turtles exposed to temperatures of 1° to 3°, (125), and conversely, that an intravenous injection of Mg causes a drop in body temperature (24, 120, 165). There was no evidence of any such (inverse) relationship between temperature and serum Mg concentration, in our dogs.

The serum inorganic phosphate concentration has been shown to rise, in hibernators (29); again, as above, no such phenomenon occurred here.

CHAPTER XVI.

RENAL FUNCTION DURING AND IMMEDIATELY AFTER PROFOUND HYPOTHERMIA.

Renal function was followed by noting the changes which occurred in the creatinine clearance (C_{cr}), urinary electrolyte protein and osmolar concentrations, urinary flow rate, TmPAH, and percentage excretion of filtered electrolytes and water, during cooling and rewarming. The relationship between the rate of reabsorption of filtered electrolyte, and the oesophageal temperature, was also studied.

Each of these expressions of renal function will be discussed, separately and serially, below.

PROTEINURIA.

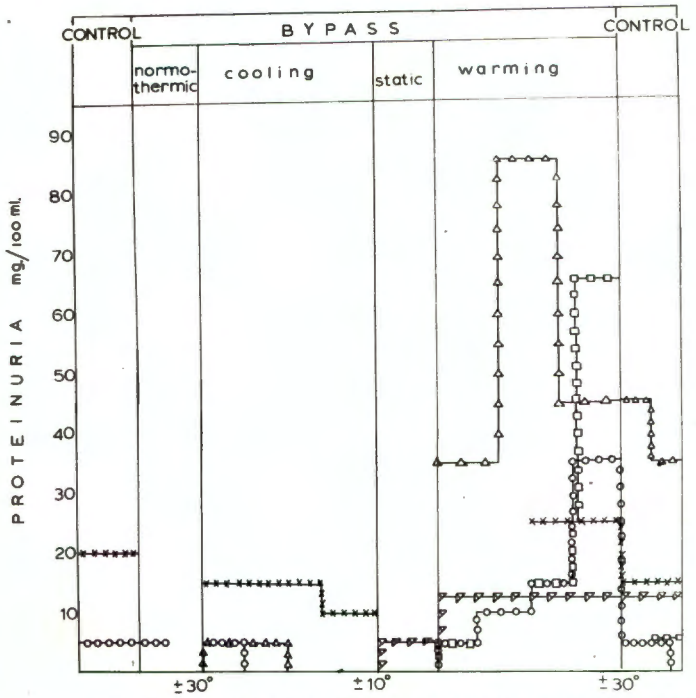
Fig. 26 summarises the degree, and time of occurrence, of proteinuria, as found in those 6 hypothermic dogs in which it was sought for, as well as in the two 'control' dogs. It is apparent that proteinuria occurs either for the first time, or in greatly increased amount, during the rewarming bypass period. Dogs A, B, and D, are particularly interesting, in that the proteinuria seen initially diminishes or even disappears on cooling, only to reappear in even greater concentration on rewarming. These data suggest that at least two factors are at work: the first, operative during cooling, preventing the appearance of proteinuria, and the other, of maximal effect during the rewarming bypass period, causing proteinuria. All the dogs developed a relative postbypass diuresis, so that the trend towards a fall in urine protein concentration at this time may be at least partly accounted for by simple dilution.

Other workers have commented on the proteinuria seen on cooling and rewarming, although their data were in no case as detailed as the above. Segar (147) after cooling dogs to rectal temperatures of 20°C by immersion in ice water, noticed a rise in urine protein concentration from a mean of 25 mg/100 ml at 20°, to 80 mg/100 ml at 37°.

There was a step-wise increase in proteinuria with rise in temperature, similar to that seen in Dogs B, D and E in Fig. 26. Hernandez and Coulson (77) found proteinuria of up to 1 G/100 ml., on rewarming chilled alligators. Harvey (74) whose studies on the cooled, perfused, isolated dog kidney have been referred to before, noticed that not only protein, but also the dye T 1824 failed to appear in the urine at low temperatures.

In the dog, it is accepted that serum albumin and globulin penetrate the normal glomerular filter, and are then reabsorbed by the cells lining the proximal convoluted tubule (100, 155). While never estimated in dogs, the protein content of the glomerular filtrate, as determined by direct micropuncture of the capsular space, has been found to be of the order of 30 mg/100 ml in amphibia (137), and 20-80 mg/100 ml in guinea pigs, and rats (112, 149, 174). We might then expect proteinuria of similar magnitude in the dog during profound hypothermia, when tubular reabsorphive mechanisms are paralysed. The absence of such proteinuria (cf Fig. 26) must mean either that protein is not normally filtered across the glomerulus; that hypothermia has not paralysed the protein reabsorptive mechanism; or that glomerular permeability to protein is lessened by cold. There is a great deal of evidence suggesting that protein is normally filtered across the glomerulus (53, 112, 137, 174);

PROTEINURIA IN HYPOTHERMIC DOGS



PROTEINURIA IN NORMOTHERMIC DOGS

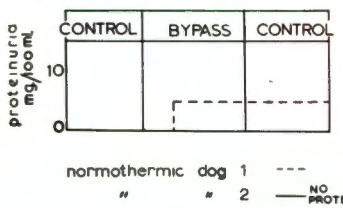


FIG. 26.

indeed it has been said that proteinuria cannot occur in animals without glomeruli (19). The paralysis of renal tubular reabsorptive mechanisms by cold will be discussed later; suffice it to say here that while inhibition of protein reabsorption during hypothermia has never been directly demonstrated, there is no reason to doubt its occurrence.

What evidence is there for diminished glomerular permeability during hypothermia? Bickford and Winton (18) noticed that haemoglobinuria did not occur at low temperatures, and thought that the cause lay in altered glomerular permeability. The tubular reabsorption of haemoglobin is however (presumably) an active process, and one could argue that its tubular transport was not as depressed by cold as was the filtered load of haemoglobin. A more cogent argument is perhaps that adduced by Anderson and Nielsen (2). These workers found that the tubular reabsorption of urea fell with cooling, until the clearance of urea approached that of creatinine. As the reabsorption of urea is a passive process, the above argument no longer holds, and they were able to conclude that tubular membrane permeability must diminish with cold.

In passing, it may be relevant to note that hypothermia diminishes the rate of diffusion of oxygen, through lung membranes (49).

Farquhar et al (53) in electron-microscopic studies of the rat glomerulus, have found the basement membrane to be an acellular gel-like structure, which contrary to earlier reports possesses no 'pores'. In their view, the basement membrane is probably dependent on both the glomerular endothelial and epithelial cells for its synthesis and maintenance, and they believe that any agent which interferes with the metabolism of either of these cell layers could conceivably affect its chemical composition and permeability characteristics.

It would seem therefore, on the balance of the evidence available, that proteinuria does not occur, or if previously present, diminishes, during profound hypothermia, as cold-induced reduction of glomerular permeability has prevented its filtration.

What of the proteinuria occurring during rewarming? As water andelectrolyte reabsorption rapidly return to normal at this time, tubular protein reabsorption presumably follows suit. The increased urinary protein content must then be due to increased glomerular permeability.

(An alternative possibility is that of tubular protein secretion; while this has been held to be the explanation for Bence-Jones proteinuria (154), it is generally believed that such a mechanism does not exist (19, 149)) Important factors increasing glomerular permeability are shock and

ischaemia. Walker et al (174) during the course of their experiments on micropuncture of mammalian glomeruli, found that "slight mechanical trauma to glomerular capillaries, far short of actual rupture, will make them permeable to gross amounts of protein". Renal ischaemia has long been known to lead to proteinuria (162, 31), and transient proteinuria is often seen in normal persons under stressful conditions such as fright, severe exercise and cold. These latter conditions are associated with a rise in urinary excretion of catecholamines (89), and release of catecholamines into the blood stream can, in turn, lead to renal ischaemia and proteinuria (93). Rennin also increases glomerular permeability to protein (149). Surgical shock is well known to lead to renal lesions of varying severity (35, 23) and "it is now widely accepted that this renal damage is due to ischaemia resulting from vasospasm of the renal vascular bed, in addition to the fall in systemic blood pressure" (39).

The surgery of cardiopulmonary bypass is certainly major enough to invoke the above as a cause of proteinuria under these conditions. Added to this is the effect of bypass itself, (cf Chapter XIV), which leads to a further marked reduction of renal blood flow. It is therefore not surprising that one of the 'control' dogs (normothermic dog 1) developed proteinuria during the bypass period

(cf Fig. 26). The proteinuria seen in the rewarming phase of initially aproteinuric, hypothermic dogs, may then simply represent an ischaemic glomerular lesion, previously hidden by the cold-induced glomerular impermeability to protein.

Extensive surgery, and cardiopulmonary bypass are not the only possible causes for renal ischemia. When the metabolism of hypothermia was first extensively investigated (21) it was stated that an oxygen deficit did not occur, and Delorme (44) believed that the reduction in oxygen demand produced by cooling was so much greater than the reduction in its availability, that a relative oxygen surplus existed at capillary level. Recent observations however have cast doubt upon this belief. While Bigelow (21), by extrapolation of his data obtained at 19°C, calculated that oxygen consumption should be zero at 10°, reports of actual observations of oxygen consumption at about this temperature, have revealed oxygen deficits of varying severity. Thus Heimbecker (76), perfusing dogs at a flow rate of 65 ml/kg/minute, at 8.5° - 12°, found oxygen deficits of from 0.6% to 20% of basal requirements. Sealy et al (145) found an 80% reduction of mixed venous blood, after an hours circulatory arrest during profound hypothermia. Kameya et al (87) have confirmed these observations. The reason for the discrepancy between these

and the earlier reports is said to be a lack of uniformity of tissue cooling produced by blood stream cooling. According to this view, while some tissues may be profoundly hypothermic, and so have a zero oxygen demand, others may be at a temperature several degrees higher, and so suffer anoxic damage. This would be the more likely to occur, as it is known (129) that cold shifts the oxygen dissociation curve to the left, thus hindering the release of oxygen.

Nor is this all. Evidence will be presented below (cf discussion on Ccr) suggesting that a state of circulatory insufficiency may arise during the rewarming of hypothermic animals; this in itself could well cause proteinuria.

SUMMARY:

The absence of the expected proteinuria during profound hypothermia, and its sudden appearance on rewarming, may be due to the interplay of two discrete factors. The first is diminished glomerular permeability produced by cold - this prevents the filtration of protein; the other is renal ischaemia, secondary to extensive surgery, extracorporeal circulation, non-uniform tissue cooling, and/or circulatory insufficiency on rewarming - this increases glomerular permeability and so leads to proteinuria.

CREATININE CLEARANCE.

The initiation of active blood stream cooling led to a prompt rise in Ccr, in dogs B, C, D, E and I. As far as I can ascertain, this phenomenon has not been previously reported, and is probably a function of our technique As pointed out in Chapter 11, no other studies of the cold kidney have as yet appeared, in which hypothermia has been induced by extracorporeal blood-stream cooling and cardiopulmonary bypass. The mechanism of the sudden change in Ccr is presumably one of cold-induced paresis of that nervous activity which had caused the preceding fall in Ccr on introduction of bypass.

Without exception, all our dogs exhibited the parellelism in fall of Ccr and body temperature, commented upon by previous workers (cf Chapter 1). Bickford and Winton (18) first observed this in 1937, and attributed it partly to the concomitant fall in arterial blood pressure, and partly to the increased viscosity of cooled blood. They demonstrated that, where the blood pressure was kept constant, a linear relationship existed between Ccr and blood viscosity, between 37° and 5°.

In two of the dogs subjected to profound hypothermia (Dogs G and I) the Ccr on rewarming, while rising slightly

above the levels obtaining at the lowest oesophageal temperatures, did not return to the control pre-bypass values, despite a rise in blood pressure. Several workers have commented upon the occasional failure of the GFR to return to control levels during the rewarming of hypothermic animals (116, 120, 146, 147) and the concensus is that this is due to a transient posthypothermic circulatory insufficiency (20, 75, 134, 139, 146, 167). Recovery has been said to occur after 1 hour (20), 3 hours (167) or 24 hours (120). Heinbecker and Swan (75, 167) postulated a too-rapid rewarming process leading to widespread peripheral arteriolar vasodilatation, with a fall in peripheral resistance and a shock-like state. It is worth noting that a normal blood pressure does not exclude the presence of a mild degree of this phenomenon - indeed, an unduly depressed Ccr maybe the only manifestation of the renal vasoconstriction responsible for the maintenance of the blood pressure (39).

The assumption underlying the above reasoning has been that no oxygen demands exist during deep hypothermia. This has since been shown not to be true (cf discussion on proteinuria), and an appreciable oxygen debt may accumulate during hypothermia. This in itself could lead to a shock-like state, with a secondary fall in Ccr.

Either or both the above mechanisms presumably underlie the failure of recovery of the Ccr in Dogs G and I.

Interestingly, acute renal failure rarely follows the use of profound hypothermia in clinical practice (9), presumably as bypass and active blood-stream cooling are induced simultaneously. It is known that hypothermia protects the kidney from acute ischaemic insult (34, 117, 142). In our dogs, active blood stream cooling was initiated only after a variable period of cardiopulmonary bypass. While this was desirable from the point of view of separating the renal effects of hypothermia from those of bypass, it is apparent that this procedure must increase the chances of inducing ischaemic damage to the kidneys. The only dog (Dog A) in which we followed the clinical practice of introducing cardiopulmonary bypass and active blood-stream cooling simultaneously, showed no reduction of Ccr on rewarming, despite 32 minutes of complete circulatory arrest during the hypothermic period.

In short, alterations in Ccr during cooling and rewarming, in our experiments, are probably more a reflection of circulatory than renal insufficiency. The main purpose served by its determination is to permit of

the calculation of the percentage reabsorption or excretion of filtered water and electrolyte.

Throughout the course of this work, the Ccr has been equated with the GFR, at all temperatures. This is in line with the practice of almost all the workers in this field. Only Segar (147) who found the clearance of glucose to be slightly higher than that of creatinine, at 20°, has queried the validity of this assumption. He suggested that creatinine might be partly reabsorbed during hypothermia. I have followed the majority ruling on this issue, however, particularly so as the clearance of creatinine has been found to be identical to that of inulin during diverse forms of renal stress (anoxia, diuretics, transport inhibitors - cf Berliner, R.W. 'The Kidney', Ann. Rev. Physiol. 1954).

As a precautionary measure, whenever PAH was given to our dogs, the C_{PAH} was calculated throughout the experiment. During profound hypothermia, tubular secretion of PAH maybe expected to be minimal or absent, and the C_{PAH} should then approximate to the GFR, or Ccr (if creatinine is not reabsorbed). This was found to be so in every case.

URINARY ELECTROLYTE CONCENTRATIONS.

The urinary Na, K, Ca, Mg and inorganic phosphate concentrations followed characteristic and constant patterns throughout our experiments. In all cases, as hypothermia progressed, urinary Na, Ca and Mg concentrations rose, while that of K fell, to approximate to their respective serum concentrations. These changes were reversed on rewarming. (In Dog B, while the urinary Mg concentration approached that of the serum, at the 'depth' of hypothermia, it reached this figure by falling from a previously higher concentration). The urinary inorganic phosphate concentration unlike the above, varied but little during cooling and rewarming, and was always much less than its serum concentration, even during profound hypothermia.

These findings are in accord with such scanty literature as exists on the subject. The best documented is the similarity between serum and urinary Na concentrations at low temperatures, an observation first made (indirectly, by estimations of the chloride) some 25 years ago - by Bickford and Winton (18). This has since been amply confirmed (4, 74, 77, 81, 146).

The renal handling of K during hypothermia has been less frequently studied. During cooling of both man and

dog to 27°-20°, the excretion of K has been variously said to rise (116), or to fall (120). More detailed studies have been made by Segar et al (146) where-in they noted a fall in urinary K concentration "to a value only three times that of serum", at 20°-22°, while urinary excretion of K rose.

In our dogs B, C, D, E and G, the urinary K concentrations fell to lower levels than any previously reported during hypothermia, so that at 10°-12°, the U/P potassium ratio lay between 1 and 2. The lower ratios obtained in our studies presumably reflect the lower body temperature attained.

These approximations of urinary Na and K concentrations, to those of serum, presumably reflect cold-induced paresis of reabsorptive mechanisms, as well as of the secretion of K. Bickford and Winton made the point that urinary Cl concentration reached serum concentration at about 18°.

Only two other investigators appear to have determined urinary Ca and Mg concentrations during hypothermia. Hernandez and Coulson (77) cooling alligators and turtles to cloacal temperatures of 6°, by confinement in a cold room, noted that "abnormal amounts" of Ca and Mg appeared in the urine.

Normally, 30% to 40% of these cations are bound to the plasma proteins; during profound hypothermia proteinura does not occur (vide supra), and urine osmolar concentration does not rise above that of serum (vide infra). The maximal urinary concentrations of Ca and Mg should then be 70% to 60% of their respective serum concentrations. Yet our studies (Dogs B, C, D, G) revealed that urinary Ca and Mg concentrations closely approached their respective total serum concentrations. The only plausible explanation for this phenomenon would appear to be the almost complete dissociation of the serum Ca-protein and Mg-protein complexes, during profound hypothermia.

During hypothermia, the ECG may develop abnormal S-T segment deflections, similar to those generated by "current of injury" potentials (126). Melrose (111) found the ECG effects of the infusion of $CaCl_2$ into dogs at both 38° and 25° to be such as to "strongly suggest" that the changes described above were due to the presence of increased amounts of ionised calcium. McMillan et al (110), cooling dogs to an oesophageal temperature of 19° by immersion in ice water, noted that the 'free' serum Ca rose from 5.0 mg/100 ml to 6.4 mg/100 ml; on the other hand the total serum Ca in their experiments also rose, from 11.4 to 14.0 mg/100 ml., so that the proportion of diffusible to

total Ca hardly changed. Similarly, Platner (132) denied any alteration in the percentage of dialysable Mg, despite a rise of 159% in the total serum Mg in goldfish and turtles exposed to cold. Both McMillan et al., and Platner, determined the free, diffusible fractions of serum Ca and Mg (respectively) by dialysis. The temperatures and pH at which these dialyses were performed are not stated; both these factors are known to affect the protein binding of Ca (173), and both might be expected to increase the percentage of diffusible Ca and Mg in the circumstances pertaining to our experiments.

Throughout our experiments, the urinary inorganic phosphate concentration varied but little during cooling and rewarming. The significance of this will be discussed later.

URINARY OSMOLALITY.

Urine and serum osmolalities were measured throughout the experiments on dogs B, C, D, G and I. In all cases the urinary osmolality fell, from the higher levels obtaining during the bypass - precooling periods, to within 9-49 mOsm/l. of the serum osmolality, at oesophageal temperatures of 10°-12°.

This finding confirms and extends other indirect and direct measurements of urinary osmolality during hypothermia. Thus hypothermic rats and ground squirrels failed to concentrate their urinary urea (78); rabbits at rectal temperatures of 22° showed a fall in the U/P inulin ratio (2); and hypothermic dogs have reduced U/P creatinine ratios (18) (147). Suk Ki Hong and Boylan, (80) measured the Uosm directly in dogs, and found that at 24°-26° the Uosm in hydropenia was reduced from 1600 to 900 mOsm/l, while after urea loading it fell from 650 to 550 mOsm/l. (These findings are all in contradiction to those of Keller (90) who could detect no impairment of osmotic flow-load curves at 28° in dogs.)

Suk Ki Hong and Boylan (80) have posited that the

fall in Uosm with reduction of body temperature follows reduction of Na reabsorption in the loops of Henle - an energy dependent process (181), and so reduced by cold - leading to a fall in the medullary osmotic gradient and so diminished removal of free water from the collecting ducts. In addition, or alternatively, ADH activity may be inhibited. ADH activity has been shown to be energy dependent in the frog skin (79); and pituitrin injections are without effect in hypothermic rats, squirrels (78, 3) and dogs (146). The circumstances of our experiments make it unlikely that endogenous ADH was present in any significant quantity, as all our experimental animals had received considerable volumes of dextrose-water intravenously. Our experimental findings therefore favour the first of Suk Ki Hong and Boylans' hypotheses on the mechanism of U_{osm} reduction in hypothermia.

In Dog C, the ureter was occluded for 3 minutes while the oesophageal temperature rose from 13.4° to 15.5°. The urine obtained immediately after release of the occlusion was of but 221 mOsm/l., (in contrast to the preceding figure of 335 mOsm/l) but rose within 10 minutes, with concomitant rewarming, to 505 mOsm/l. The passage of hypotonic urine, after relief of urinary obstruction, is a well recognised phenomenon, both clinically (28, 180, 140, 51) and experimentally (83, 182). Various explanations

have been offered. Jaenke and Bray (83) believe the concentrating defect is secondary to an increased hydrostratic pressure within the renal pelvis. Bricker et al (28) attribute it to a defect in proximal tubular Na transport, leading to an osmotic-diuresis-like effect. The best experimental analysis of the problem appears to be that by Abbrecht and Malvin (1). These workers based their experimental design upon the mathematical treatment of the countercurrent multiplier system by Hargitay and Kuhn (71), wherein it was concluded that the maximum possible U/P osmolar gradient depends upon the rates of flow of both urine and blood through the renal medulla. Abbrecht and Malvin found that, while a fall in GFR leads to a rise in Uosm (where RPF is held constant), a still greater reduction in GFR causes a fall in urinary osmolality. This they felt was a reflection of the fact that now the medullary blood flow was relatively large compared to urine flow, and so the peritubular capillaries by removing Na, limit the development of a medullary Na gradient.

The isolated - and accidental - observation on Dog C is of interest in relation to the above. It can be stated categorically that intrarenal pelvis pressure did not rise substantially on occlusion of the ureter; indeed the attempt at a 'stop-flow' experiment was abandoned

for this very reason. This would seem to invalidate the explanation proposed by Jaenke and Bray. Secondly, the phenomenon appeared after occlusion at a markedly subnormal body temperature (the mean oesophageal temperature during the post-occlusive urine collection period was 18.1°), when the efficacy of tubular Na transport was still substantially below control levels (17.5% of the filtered Na was excreted during this period, as compared to 1.7% in the following urine collection period). Finally, the speed of recovery is impressive; within 10 Minutes the urinary osmolality - and therefore presumably that of the medullary interstitium too - was 505 mOsm/l.

Urine specimen No. 11, in Dog C, had an osmolality of 403 mOsm/l., this was 84 mOsm/l higher than that of urine specimen 10, and 64 mOsm/l higher than that of sample 12. The figure was checked several times. Assuming the absence of a technical error, the only explanation appears to be that this fleeting rise in Uosm reflected a sudden transitory fall of medullary blood flow, in the presence of tubules still actively pumping Na into the medullary interstitium. The mean oesophageal temperature during this urine collection period was 14.5°. Unfortunately the systolic arterial blood pressure,

which might have provided confirmatory evidence for this view - was not recorded. The C_{cr} however hardly changed from that present during urine collection period 10.

No similar instance of a rise in Uosm during hypothermia was seen in the remainder of our experiments.

URINARY FLOW RATE.

Many of the earlier papers dealing with renal function during hypothermia make mention of a rise in urine volume on cooling. A brief comment on this phenomenon seems appropriate at this point.

The urine flow rate (volume of urine produced per minute) is not, of course, a primary feature of renal function but rather the resultant of the rates of glomerular filtration and of the tubular reabsopption of water. The discrete effects of hypothermia on these two factors are discussed in detail elsewhere.

The interplay of variations in GFR and the renal reabsorption of water, to produce changes in urinary flow rate, is clearly delineated during the experiments on Dogs, B, C, D, E, G and I. During normothermic, or mildly hypothermic, bypass the GFR falls, while the renal tubular reabsorption of water remains relatively unimpaired. The urinary flow rate consequently falls markedly. On introduction of active blood stream cooling, the Ccr (partly) returns to pre-bypass levels, while water reabsorption is minimally impaired. The urinary flow rate therefore increases, or rather, returns toward prebypass levels. As cooling progresses, the Ccr falls

again, while water reabsorption becomes less and less efficient; according as to which factor is the greater, urinary flow rate falls (Dog B, D) or rises (Dogs C, E, G, I). At oesophageal temperatures of 10°-15°, the reduction of Ccr is so great that even with zero water reabsorption (never achieved during our experiments) the urinary flow rate must fall to low levels.

Suk Ki Hong, and Boylan (80) cooled both hydropenic and urea loaded dogs to rectal temperatures of 24°-26°, by immersion in ice water. A slight increase in urine flow rate resulted, and in both groups of dogs, the increase was numerically equal to the simultaneously observed reduction in free water clearance ($T^{c}_{H_2O}$). That this is not the whole explanation of the mechanism of cold-diuresis is apparent from inspection of the data relating to any of the dogs presented here, in which urine flow rates rose on cooling (Dogs C, E, G, I). Taking Dog C as a typical example, (Table 4) it can be seen that between urine collection periods 6 and 9, the urine flow rate rose by 1.98 ml/min., yet the $T^{c}_{H_2O}$ fell by only 0.69 ml/minute.

The reason for the discrepancy between the observations of Suk Ki Hong and Boylan, and those presented here, is simply that in their experiments cooling was not marked

enough to reduce the U_{osm} to near serum levels. Their observations were correct, as far as they went, but their conclusion applies only to the initial phase of cold-diuresis. On further cooling, proximal tubular reabsorption of isotonic glomerular filtrate fails, and this causes the further rise in urinary flow rate seen in our experiments.

TABLE 4.

The rise in urinary flow rate during hypothermia is greater than the simultaneous fall in the free water clearance ($T^{c}_{H_2O}$) and occurs despite a fall in the creatinine clearance (C_{cr})

DOG C

URINE SAMPLE	6	7	9
Urine flow rate (ml/min)	2.26	3.54	4.24
$T^{c}_{H_2O}$ (ml/min)	0.85	0.30	0.16
C_{cr} (ml/min)	14.5	11.7	10.5
Mean Oesophageal Temperature ^{o}C	25.9	25.6	23.0

$T^{c}_{H_2O} = C_{osm} - V$, where $T^{c}_{H_2O}$ = free water clearance.

C_{osm} = osmolar clearance

V = urine volume /minute

$C_{osm} = \frac{U_{osm} \times V}{P_{osm}}$, where U_{osm} = urinary osmolality

P_{osm} = plasma osmolality

(cf ref. 159 for the derivation of these formulae).

TmPAH.

PAH was administered intravenously to dogs B, C, D, E, G and I, as well as to 'Normothermic Dog 1'. Gross fluctuations in serum concentrations of PAH rendered the calculation of the TmPAH impossible, in Normothermic Dog 1 and Dog G; the serum samples of Dog E were mislaid before their PAH content could be determined. The ERPF was measured in Dog I. The TmPAH was therefore measured only in Dogs B, C and D.

In these three dogs, the pre-bypass TmPAH was within normal limits (cf Appendix C) prior to the fall in body temperature. During profound hypothermia, the TmPAH fell to very low levels, achieving negative values in Dogs B and C. On rewarming, Dog C transiently regained its prebypass TmPAH, but this soon fell; after resumption of normal circulation, the TmPAH, while greater than during profound hypothermia, was still considerably below the 'control' prebypass level in all three dogs.

Urinary PAH concentrations fell to a low plateau during cooling; but rose again promptly on rewarming, in all the 7 dogs in which it was measured. At its lowest, the urinary PAH concentration was always considerably above that of the plasma. The lowest U/P PAH ratios were

achieved by Dogs B and C, at 11°-12°; these were 2.3 and 1.75 respectively.

By and large, these observations are in accord with such data as are available in the 'literature'. Measurements of TmPAH in man (116) and dogs (90, 22, 127), cooled to 23°-28°, have revealed marked reductions with a falling body temperature; both Keller (90), and Blatteis and Horvath (22) remark on the equisite thermal sensitivity of the renal transport system for PAH: Forster (58) gives a Q_{10} of 2 for PAH secretion in the fish.

Studies on isolated perfused dog kidneys (74), and on renal tissue in vitro (58, 60, 38) confirm this cold-inhibition of PAH transport. Harvey (74) noted that PAH transport was more cold sensitive than that of Na; and by extrapolation of his data concluded that the U/P PAH ratio should approach unity at circa 4°.

A negative TmPAH implies more tubular reabsorption than tubular secretion, of PAH. Foulkes and Miller (60) found that, while non-metabolising kidney cells were impermeable to PAH, intraluminal PAH could diffuse, passively, into the medium (extra cellular fluid). On the other hand, metabolically active renal cells concentrated PAH to high levels intracellularly; when these cells were then exposed to cold, the rapidly

diffusible fraction of the intracellular PAH ran out into the medium. These two processes - diffusion of PAH from the tubular lumen to the E.C.F., and run-out of rapidly diffusible intracellular PAH into the tubular lumen, - must then oppose each other, during profound hypothermia. To add to the difficulty of interpretation of our findings, is the demonstration by Asheim et al (6) of occasional negative TmPAH values even in normal dogs. These considerations, I believe make it impossible to evaluate the role of hypothermia, in the development of negative TmPAH values, in our experiments.

In all 3 dogs, the TmPAH was considerably lower after the bypass-hypothermia period, than before. While the wide range of normal given by Asheim et al (6) makes the determination of TmPAH valueless, as an absolute estimate of renal functional integrity, the comparative decline after hypothermia suggests tubular secretory insufficiency. It has already been pointed out (cf discussion on proteinuria) that there is reason to believe that a state of mild tissue anoxia with or without some circulatory insufficiency, exists at this time. I believe these factors underlie the fall in TmPAH seen here.

PERCENTAGE EXCRETION OF FILTERED WATER AND ELECTROLYTE.

The data on percentage excretion of filtered electrolyte and water, during cooling and rewarming, reveal the following:

(1) The percentage excretion of filtered Na, varied roughly in parallel to that of water, particularly at the lower temperatures.

(2) Although the minimum oesophageal temperature achieved (10^{o}-13^{o}) was much the same in almost all the dogs (Dog K was cooled to 6^{o}), the greatest cold-induced excretion of filtered water and electrolyte varied markedly, from dog to dog. Thus, for example, Dog D excreted only 13% of filtered Na, at a mean oesophageal temperature of 12.2^{o}, while in Dog E this figure was 73% at 11.5^{o}.

(3) The rise in percentage excretion of Na, water, Ca, Mg and inorganic phosphate, on cooling, was paralleled almost exactly by the fall in excretion on subsequent rewarming. In Dogs D (particularly) and G, however, the percentage excretion at any given temperature was a little higher on rewarming than on cooling.

(4) Unlike the case for the other electrolytes, and water, the variations in percentage excretion of "filtered" K, with changing temperature, did not follow a regular pattern. While there was a trend to a higher excretion rate at lower temperatures, the graphic plot of

percentage excretion against temperature yielded a wide scatter of points.

(5) The rise in percentage excretion of filtered inorganic phosphate, with cooling, was very markedly less than the simultaneous rise for the other electrolytes, and water. This was true in every one of the four dogs (dogs B, C, D, G) in which it was determined.

Each of the above merits some comment.

The rise in percentage excretion of filtered Na, with cooling, reflects the progressive impairment of two discrete processes: (1) Na reabsorption against a concentration gradient, in the distal tubule (2) isosmotic proximal tubular Na reabsorption. It is apparent from our data that the former is the first to go. Thus, for example, in Dog B the urinary Na concentration was 132 meq/l. (urine collection period 6) when the plasma Na concentration was 142 meq/l, yet only 26% of the filtered Na was excreted. Once the urinary Na concentration approximates to that of the plasma, the percentage excretion of Na and water must necessarily be the same. This observation is not new. Bickford and Winton commented 25 years ago on the identity of urine and plasma chloride concentrations below 18°, and others have found the same to be true of Na (74, 77, 81, 146).

At 37°, and during mild hypothermia, only small percentages of filtered Na and water are normally excreted, when on extracorporeal cardiopulmonary bypass, at the flow rates used in this study (cf Chapter XIV). While the urinary Na concentration is low, and its percentage excretion therefore appreciably lower than that of water, this difference is insignificant in comparison to the vast increases brought about by cooling.

These considerations explain the apparent parallelism in percentage increase of Na and water excretion on cooling. At the upper end of the curve, the figures would be expected to be identical; at the lower, differences exist but are comparatively slight. Close inspection of figures 4, 11, 14, 17 and 20 will confirm this statement.

Controversy has existed for several years, as to the nature of the process of renal proximal tubular reabsorption of filtered Na and water. Many believed this to be a passive transport process, simply a response to the raised colloid osmotic pressure in the peritubular capillaries (71, 27). This view seemed to be substantiated by the studies of Andjus (3), and Segar et al (146), who showed respectively, that on cooling rats and dogs to rectal temperatures, of 20°, the renal reabsorption of filtered Na and water fell by only 12% of that filtered; this they believed, implied that

the reabsorption of the remaining 88% was cold-insensitive, and hence mediated via a passive transport process. Malvinet al (107) calculated, on the basis of stop-flow experiments on dogs, that the reabsorbed fluid had the same Na concentration as, and was therefore hypo-osmotic to, plasma; this was cited as confirmatory evidence of the above.

Others however would have none of this (for an authoritative review of indirect contradictory evidence, see Leaf 102). Schatzman et al (141) working with single proximal tubules of Necturus showed that Ouabain and dinitrophenol inhibited the reabsorption of proximal tubular fluid. Whittemburg et al (177) demonstrated not only that the osmotic pressure generated by the plasma proteins within the peritubular capillaries could account for but 1% of the reabsorption of proximal-tubular fluid, but also that the movement of Na from tubular lumen to renal interstitium took place against an electrochemical gradient (36). This proved the active nature of the transport process. Lassen et al (99) found that renal oxygen utilisation varied linearly with the quantity of Na reabsorbed by the kidney, and so implied the existence of an oxygen dependent active transport process for Na reabsorption.

Our results are in accord with the latter view i.e. favour an active transport process. Cooling to 10^{0} resulted

in the excretion of 10% to 73% of the filtered Na and water, far more than could be derived from paralysis of only distal tubular function. Had Andjus (3) and Segar et al (146) cooled their animals further, to 10° rather than to 20°, they would presumably have obtained figures similar to ours.

At comparable mean oesophageal temperatures (10° to 13°) the percentage excretion of filtered Na in dogs B, C, D, E, G, I and L ranged between 13% and 73%. That of Dog K, was 66.7% at 6°. Two possible explanations for this brdad scatter come to mind. Throughout this Thesis, an implicit assumption has been that renal metabolism is directly geared to renal temperature. This may simply not be true. Alternatively, temperature gradients between oesophagus and kidney, or between various parts of the kidney, may conceal this relationship. I believe the latter explanation to be the correct one.

There is a good deal of evidence to support this contention. In dogs I, J, K and L, the simultaneous oesophageal and renal temperatures (the latter measured by means of a single Thermistor probe plunged at random into the substance of the kidney) were found to differ markedly and unpredictably on cooling (Figs. 24, 25). While the renal temperature always fell more rapidly than did the oesophageal, it could be higher (Dog I), lower (Dog L) or identical

(Dogs J, K) to the latter, at the 'depth' of hypothermia. Other workers (152, 5, 153, 72) have found large temperature gradients to exist between various organs and tissues during hypothermia. There may even be appreciable temperature differences between various sites within a single organ; this has been shown to be true for the heart (55) and the brain (187). Finally, while Levy (104) found that temperatures taken at 5, 10 and 15 mm below the capsule were identical in the cold, isolated and perfused kidney, large intrarenal temperature gradients have been demonstrated in both man (91) and dog (91, 56).

Interestingly, Fisher et al (56) showed that the renal cortex cooled more slowly than did the medulla, and that the more rapid the cooling process, the greater the temperature difference between these sites. They postulated that pre-cortical arterio-venous shunts come into effect during hypothermia, so that total kidney perfusion may not occur.

It is apparent that if factors such as these were present during our experiments, we could hardly hope to demonstrate consistent rates of water and electrolyte excretion, at comparable temperatures, in different dogs.

The percentage excretion of "filtered" Ca and Mg was calculated on the assumption that the serum content of each

of these cations is completely diffusible. This is of course not so, but if the proportion of protein-bound to total cation remains constant during cooling and rewarming, then the percentage excretion as calculated above should vary in parallel with the percentage excretion of the truly diffusible fractions of the serum Ca and Mg. Evidence has already been presented (cf section in this Chapter on urinary Ca and Mg concentrations during hypothermia) suggesting that in fact the protein-bound fractions of these cations diminish considerably during cooling. In that case, the figures of percentage excretion of filtered Ca and Mg at low temperatures - circa 10^{0} - 12^{0} - are meaningful, and absolute. Conversely, as there is no reason to doubt the normal protein-binding at near-normal body temperatures, the figures of percentage excretion at the lower end of the curves (figs. 4, 11, 14, 20) are, in an absolute sense, too low. The net result is that the true curves, for percentage excretion of filtered Ca and Mg, plotted against temperature, would have a slightly lesser slope than those depicted in figures 4, 11, 14 and 20.

Normally, all the filtered K is believed to be reabsorbed in the proximal tubule while in the distal tubule, K is secreted in exchange for Na (14). During hypothermia, the quantity of Na available for exchange increases, as its

distal tubular reabsorption diminishes. On the other hand, one imagines that the secretion of K will diminish too, as will the proximal tubular reabsorption of filtered K. The variations in percentage excretion of "filtered" K, during progressive body cooling, will then reflect the increasing impairment of at least three distinct mechanisms. If a differential cooling rate between medulla (K secretion and exchange) and cortex (K reabsorption) (56) is added, the wide scatter of points of calculated percentage excretion of "filtered" K against temperature, is perhaps not surprising.

Comparison of the rise in percentage excretion of filtered Na, water, Ca, Mg, and inorganic phosphate, on cooling, with the subsequent fall on rewarming, was made initially in an attempt to seek evidence of cold-induced renal damage. Dog G, and particularly Dog D, seemed to provide such evidence, as on rewarming the rate of excretion of filtered solute and water, at any given temperature, was greater than it had been on cooling. However, there is reason to believe that the experimental procedure adopted was conducive to the development of at least anoxic renal damage (cf discussion on proteinuria, and on the creatinine clearance, during hypothermia); consequently, the part played by hypothermia in bringing about these changes cannot be assessed from our data.

The percentage excretion of filtered inorganic phosphate like that of the other electrolytes and water, rose with cooling, but to a considerably lesser extent. Also, the relationship between its rate of excretion, and the oesophageal temperature, was a linear one, in contrast to the curvilinear regression lines obtained for the other electrolytes and water. This phenomenon has been observed before; Segar(147) cooling dogs to rectal temperatures of 20° by immersion in ice water, noted that while the percentage excretion of filtered water rose twenty fold (from 0.4% to 8.8%), that of inorganic phosphate increased less than three fold (from 12.2% to 30.5%). He made the comment that this degree of failure of phosphate reabsorption was of the same order of magnitude as that seen in hyperparathyroidism, and concluded that while both cold and hyper-parathyroidism are ineffective in altering isosmotic phosphate reabsorption, both inhibit the enzyme systems) responsible for the reabsorption of additional phosphate. That this explanation is inadequate is apparent from the findings in dogs B, C, D and G, where it is seen that phosphate reabsorption is little affected even where the isosmotic reabsorption of filtered Na and water is grossly defective. An alternative explanation for this phenomenon is given below.

TEMPERATURE COEFFICIENTS AND APPARENT ACTIVATION ENERGIES OF THE RENAL REABSORPTION OF ELECTROLYTES.

The method of calculation of the apparent activation energies for the renal reabsorption of Na, Ca, Mg, and inorganic phosphate has been described in Chapter X; the theoretical basis of the concept of 'Activation Energy' is briefly expounded in Appendix E.

The temperature coefficient (Q_{10}) is the ratio of the speed of a reaction at one temperature, to that at a temperature 10°C lower. The reaction speed has been obtained here in the same way as for the activation energy calculations, i.e. absolute rate 'corrected' for variations in filtered load.

Both temperature coefficients and apparent activation energies for the renal reabsorption of Na, Ca, Mg and P (as found in Dogs B, C, D, E, G, K and L) are given in Table 5. So far as the conclusions that will be drawn from these data are concerned, temperature coefficients alone would have sufficed; however the intellectual considerations, and experimental potential, underlying the concept of 'activation energy' are so much richer than the bald statement of temperature coefficient, that these are given too.

Inspection of Table 5 reveals a wide range in the

calculated apparent activation energies for the renal reabsorption of any one solute. Thus, for example, that of Na varies between 1,300 cals/degree and 14,020 cals/degree. The reason for this broad variation from dog to dog in what might have been expected to be a constant figure, is presumably identical to that advanced for the variations in percentage excretion of filtered solute, viz: nonparallelism in rates of cooling of oesophagus and kidney, as well as the occurence of intrarenal temperature gradients. The highest figures are probably the most accurate.

As a consequence of this, no conclusions can be drawn from the calculated apparent activation energies, of the renal reabsorption of Na, Ca, Mg and inorganic phosphate, other than: (1) the apparent activation energy of Na reabsorption is at least 6700 cals/degree, and probably nearer to 14,000 cals/degree: (2) Ca and Mg reabsorptive processes have lower apparent activation energies than that of Na; this is all the more likely to be true as it was pointed out in a previous section that the real slope of Ca and Mg reabsorption curves versus temperature is lower than that calculated (due to the protein binding of the divalent cations Ca and Mg at near normal body temperatures). (3) the apparent activation energy for inorganic phosphate reabsorption is very markedly less than those for Na, Ca, or Mg reabsorption, and probably

never exceeds 2000-3000 cals/degree.

This last is of interest. An activation energy of less than 5000 cals/degree, or a Q_{10} closely approximating to 1.03-1.04, is suggestive of a purely physical process such as diffusion (173). Our data therefore suggests that the renal tubular reabsorption of filtered inorganic phosphate might be mediated via a passive transport process, i.e. one requiring no expenditure of metabolically derived energy. At first sight, this is a startling concept. The proximal renal tubular cells - the site of phosphate reabsorption (131) - are veritable powerhouses of metabolic energy, and partake in a multiplicity of chemical reactions. It seems inconceivable that inorganic phosphate, which might be expected to participate in diverse phosphorylation reactions can diffuse passively through such cells. Yet a number of isolated observations can be culled from the literature, which when taken together, tend to support this concept.

The metabolic inhibitors 2:4 dinitrophenol (121), phlorizin, Benemid, and mercurial diuretics (159) are all without effect on tubular inorganic phosphate reabsorption. In the cat (69), and the parathyroidectomised rat (42) the rate of filtration of inorganic phosphate closely parallels the rate of its tubular reabsopption (expressed in mg phosphate per 100 ml. of glomerular filtrate). Similarly,

TABLE 5.

Temperature coefficients‡ (Q10) and Apparent Activation Energies* (E) of the renal reabsorption of Na, Ca, Mg and inorganic phosphate.

DOG	Na		Ca		Mg		P	
	E	Q10	E	Q10	E	Q10	E	Q10
B	2,740	1.17	916	1.05	3340	1.21	555	1.03
C	8940	1.64	8260	1.60	5920	1.38	1010	1.06
D	1339	1.08	1092	1.07	973	1.05	121	1.01
E	14,020	1.98	-	-	-	-	-	-
G	4240	1.29	8830	1.63	4040	1.30	513	1.04
I	10,250	1.80	-	-	-	-	-	-
K	8950	1.65						
L	3630	1.25	-	-	-	-	-	-
Means	6764	1.46	4775	1.34	3568	1.24	550	1.04

‡ Temperature Coefficients (Q10) measured between 15° and 25°C

* Apparent Activation Energy (E) is in calories/degree.

in dogs, while the rate of tubular reabsorption of phosphate falls with a falling glomerular filtration rate, the reabsorption expressed as a percentage of that filtered, remains constant (73). Experimentally produced nephritis (by amino-nucleoside poisoning, infection, or anti-kidney serum) produces no alteration in the renal handling of inorganic phosphate (135).

The urinary excretion of inorganic phosphate increases in acidosis (130). Thus a marked phosphaturia is seen during sleep, as well as after NH_4Cl administration (95, 73). Conversely, alkalosis diminishes the degree of phosphaturia. This relationship of urinary phosphate excretion to pH is perhaps explicable if one postulates that its proximal tubular reabsorption occurs by non-ionic diffusion. The pK_2 of urinary phosphate varies between 6.5 and 6.9 (144); perhaps the slight fall in pH of the glomerular filtrate in acidosis produces a large enough increase in ionisation of inorganic phosphate to account for its greater urinary excretion.

Two intrinsic renal disease states characteristically manifest gross distubbances of phosphate excretion. In Lignac-Fanconi disease, the majority of filtered phosphate is excreted; yet here precisely that part of the proximal tubule known to be involved in phosphate reabsorption is

characteristically anatomically deformed (43) and one can readily postulate an abnormal physico-chemical impermeability to phosphate. In pseudohypoparathyroidism, on the other hand, almost all the filtered phosphate is reabsorbed (48), and this without any evidence of glandular or metabolic overactivity.

In summary, there is a good deal of experimental fact in accord with, or at least not against, the concept of a passive transport process in the renal reabsorption of filtered inorganic phosphate. While I have indulged in a little speculation, I believe this idea worthy of further investigation. It is noteworthy that the temperature coefficient of phosphate transfer - probably as HPO_4^{--} - in the red blood cell (68) is high. This is precisely the opposite to our findings for the kidney, and perhaps, if our observations are confirmed, would argue in favour of a specialised membrane structure to the proximal renal tubular epithelium.

The literature contains few estimates of the apparent activation energy of the renal reabsorptbn of Na, and none at all for the reabsorption of Ca, Mg and inorganic phosphorus. Cort and Kleinzeller (40), working with dog kidney cortex slices, estimated the apparent activation energy of Na extrusion at 12,600 cals./mol. Harvey (74),

perfusing isolated dog kidneys at varying temperatures, noted that Na and H_2O reabsorption fell roughly in parallel, but irregularly, with cooling. I believe the irregularity was more apparent than real, and due to his failure to correct these absolute reabsorption rates for variations in filtered load. Calculating the apparent activation energies of Na and H_2O reabsorption from his data, one obtains values of approximately 16,300 and 12,150 cals/degree, respectively. The results obtained by Cort and Kleinzeller, and Harvey, approximate to our figure of 14,000 cals/degree, given above as probably the most likely to be correct.

Hoshiko, (81) perfusing isolated bullfrog kidneys, found the apparent activation energy of Na reabsorption to be of the order of 29,000 cals./mol., a very different figure to those quoted above. However, he also noted that in his preparation, the reabsorption of Na, at any given temperature, was not constant but fell progressively with time. This phenomenon has never been noted in the dog, and the reabsorptive processes in bullfrog and dog may therefore perhaps not be directly comparable.

CHAPTER XVII.

A NOTE ON RENAL EFFICIENCY.

In 1931, Borsook and Winegarten (26) published a still-quoted paper, where-in they calculated the efficiency of the kidney as 1%. Our findings on renal function during hypothermia have led me to review this calculation.

When their paper appeared, over 30 years ago, the concept of a renal filtration-reabsorption mechanism was still controversial. They based their calculations of renal efficiency on what then seemed an unassailable assumption, viz. (their phraseology): "The function of the kidney is to produce urine". Urine differs from plasma most strikingly in that many of its constituents are of a totally different order of concentration. The minimal free energy needed to produce a concentration change, for any given solute, can be calculated using the equation:

$$-\Delta F = RT \ln \frac{\text{conc.}_p}{\text{conc}_u}$$

where $-\Delta F$ = free energy change, measured in calories.

R = Gas constant

T = absolute temperature

Conc._p and Conc._u = plasma and urinary concentrations of the solute in question.

It followed that "The minimal (renal) work could be considered as a quantity equal to the sum of the free

energy changes for the transport of each constituent, including water, from blood to urine". They then calculated the efficiency of the kidney by comparison of the total free energy change as derived above, with the energy actually utilised by the kidney, as observed experimentally.

These calculations need revision in the light of todays knowledge of the mechanism of urine production. The process of glomerular filtration is dependent on the height of the arterial blood pressure, and thus on work done and energy utilised by the heart and circulation. Without this, no urine can be formed. If this additional energy drain is included in the calculation, the estimated efficiency of the kidney (thought of as a urine producing organ) drops even further, to approach vanishing point.

The kidney expends energy only on the relatively slight modification and subsequent reabsorption of 99% of the glomerular filtrate. Should it be atraumatically paralysed by some means, so that it does no work whatever, all the glomerular filtrate should then appear as urine. This in fact is seen during experimentally induced profound hypothermia - as shown in this Thesis - when the renal tubular reabsorption of Na and water (expressed as a percentage of that filtered) falls progressively with

falling temperature (cf Fig. 26). In cooled, isolated and perfused kidneys, the volume of urine produced increases many times, and varies directly with the height of the arterial blood pressure (18).

Similarly, Lassen et al (99) have found that the renal oxygen uptake correlates closely with the amount of Na reabsorbed from the glomerular filtrate, and concluded that Na reabsorption represents the main work of the kidney.

The validity of Borsook and Winegartens computation rests on the assumption that "the function of the kidney is to produce urine". Consideration of the above however suggests that it is a function of the kidney NOT to produce urine. This paradox is rendered more intelligible by recalling the present concept of renal evolution wherein the migration of the "protovertebrate" (158) from an aquatic to a terrestial habitat was paralleled by the development of salt and water retaining kidneys.

The usual definition given today of renal function is that of the regulation and maintenance of the physicochemical composition of the extracellular fluid. The kidney does this "in reverse" (158) by reabsorbing what is needed from, and secreting what is unwanted into, that fluid which the action of the heart and circulation would otherwise discard from the body, i.e. the glomerular filtrate. Renal

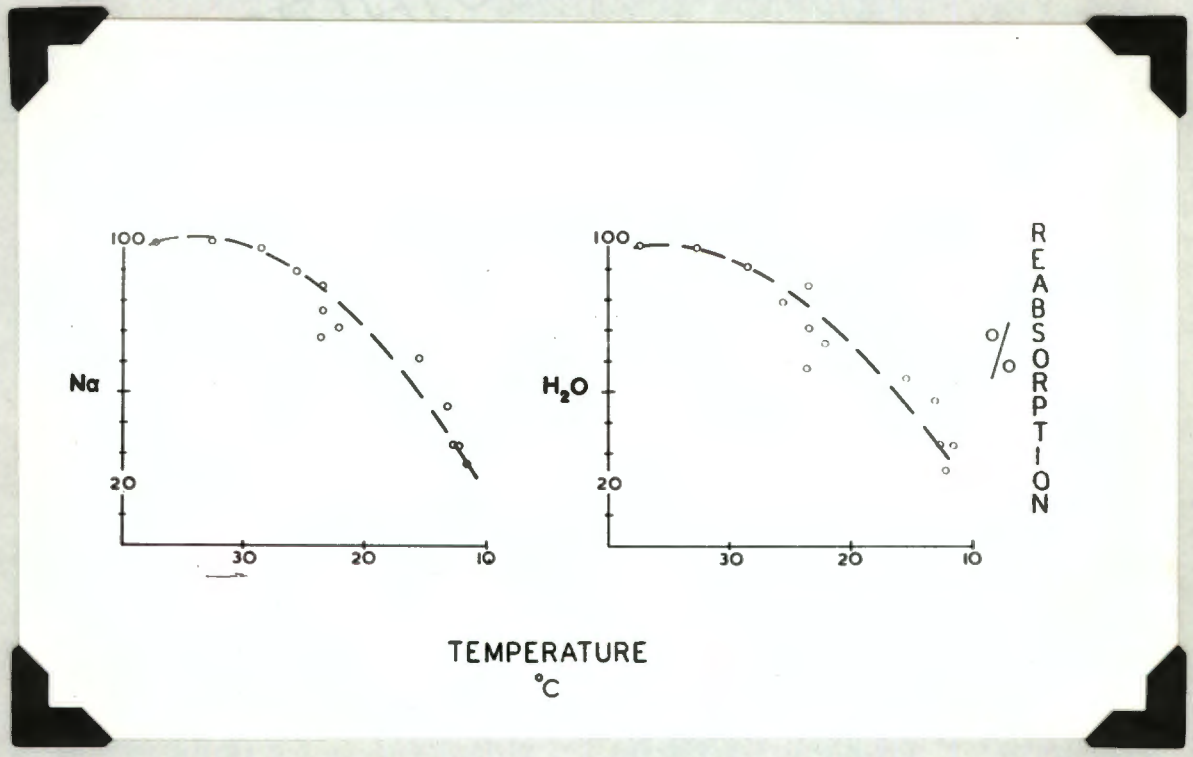


Fig. 27

Legend: The data for this figure are derived from the experimental findings on Dog E, and are simply the inverse of those plotted in Fig. 17.

efficiency must therefore be calculated by comparing the theoretical minimum energy needs of such reabsorption and secretion with the experimentally observed energy utilisation of the kidney.

It would seem therefore that the modern interpretation of Borsooks and Winegartens calculations is not that the efficiency of the kidney is 1%, but simply that a minimum of about 1% of the energy used by the kidney is spent on altering water and solute concentrations in the 'average' urine.

CHAPTER XVIII.

SUMMARY AND CONCLUSIONS.

There have, in the past, been few studies of renal function in the intact animal, and none at all in man or dog, at body temperatures below 20°C. In the latter, cardiac arrest was the invariable barrier to further cooling. The use of an extracorporeal cardiac bypass device has overcome this difficulty, and warm-blooded animals can now be cooled to 1°, with subsequent recovery. The work presented in this Thesis is the first to make use of this advance in technique, to study renal function in the intact dog during profound hypothermia.

Ten dogs were used to observe variations in renal function during and just after the induction and maintenance of profound hypothermia. The dogs were rendered hypothermic by means of a modified Dewall-Lillehei helix-reservoir bubble oxygenator, into which a heat exchanger had been incorporated. Two additional dogs were subjected to cardiopulmonary bypass alone, without active cooling, to serve as controls.

A number of facts of physiological interest have emerged from these experiments.

During Normothermic Extracorporeal Cardiopulmonary Bypass:

The GFR falls despite a well-maintained arterial systolic blood pressure; urinary osmolality rises; urinary Na, Ca and Mg concentrations fall; and the urinary K concentration is unchanged or relatively little depressed. The percentage excretion of filtered water, Na, Ca and Mg falls, while that of K is unaffected.

In states of shock or haemorrhage, the labile vasomotor system of the kidney is generally thought to play an important part in the maintenance of the systemic blood pressure. The fall in GFR, (in the presence of a normal blood pressure) while on bypass, is in accord with this concept.

The changes in urinary electrolyte and osmolar concentrations, and percentage excretion, are interpreted as reflecting the effects of unimpaired renal tubular function upon a much reduced volume of glomerular filtrate.

During Hypothermic Extracorporeal cardiopulmonary bypass:

The serum Na concentration drops, and serum osmolality rises. These changes reflect our use of intravenous (hypertonic) 10% dextrose-water infusions : during hypothermia, glucose is not metabolised, the E.C.F. osmolality rises, and water is drawn from the intracellular fluid to lower the serum Na concentration by dilution.

In relatively normothermic dogs, on bypass, the

initiation of active blood-stream cooling produces a sudden rise in Ccr. This presumably is due to cold-induced paresis of that nervous activity which had previously lowered the GFR upon introduction of normothermic bypass. Further cooling leads to a marked drop in Ccr, in parallel with a fall in the systemic blood pressure; this has been seen repeatedly by other workers in the past.

Proteinuria, where present, diminishes or disappears during profound hypothermia. It then reappears, or appears for the first time, in greater amounts during rewarming. This phenomenon is probably the resultant of the interplay of two discrete factors, viz. (1) decreased permeability of the cold glomerular membrane to protein, and (2) mild tissue anoxia, arising either during the period of maximal cooling, or during the rewarming phase.

Urinary osmolar, Na, K, Ca and Mg concentrations approximate to those of serum, at 12^{0}-10^{0}. This must reflect the paresis of tubular mechanisms for reabsorption of these electrolytes and water, as well as of the secretion of K. The fact of urinary Ca and Mg concentrations approximating closely to their respective serum concentrations, suggests the almost complete dissociation in the cold of the normally occurring serum Ca-protein and Mg-protein complexes.

It is noteworthy that the urinary inorganic phosphate concentration changes but little with cooling, and even at 10°, is far from approaching that of serum.

The percentage excretion of filtered water, Na, Ca and Mg rises on cooling. That of filtered inorganic phosphate rises too, but to a strikingly lesser degree. These increases, like the alterations in their urinary concentrations, must be a reflection of the cold-induced paresis of their reabsorptive mechanisms. There is a tendency for K excretion, calculated as a percentage of that filtered, to rise with cooling; unlike the other electrolytes however it is not possible to derive a consistent relationship between K excretion and body temperature, in any given dog.

Two factors determine the urinary flow rate: the GFR, and the rate of tubular reabsorption of filtered water. The latter is related, to the percentage excretion of filtered water, per minute. During cooling, these two factors vary in opposite directions; as the GFR diminishes, the percentage excretion per minute of filtered water, rises. In consequence, depending upon small individual differences, urinary flow rate may increase, decrease or remain unchanged during the induction of hypothermia.

Although all the dogs showed a rise in percentage excretion of filtered water and solutes on cooling, the final percentages attained at comparable temperatures, were strikingly dissimilar from dog to dog. Similarly, the rate of change of percentage excretion per degree of temperature (expressed either as the apparent activation energy, or Q_{10}, for each solute) varied widely from dog to dog. Throughout these studies the oesophageal was taken as the reference temperature. The above inconsistencies argue in favour of both oesophageal-renal, and intra-renal, temperature gradients. Direct measurement showed that not only are oesophageal and renal temperatures in a given dog not necessarily identical, but also that the relationship of one to the other may vary.

Temperature coefficients and apparent activation energies of the renal reabsorption of Na, Ca, Mg and inorganic phosphate were calculated. Due presumably to the oesophageal-renal, and intra-renal, temperature gradients referred to above, wide variations in these parameters were found in different dogs. The single permissible quantitative conclusion appears to be that the tubular mechanism for inorganic phosphate reabsorption has an apparent activation energy of considerably less than 5000 cals./degree; this argues in favour of its being

mediated via a passive transport process.

On rewarming, the changes in urinary electrolyte concentrations, and of the percentage excretion of filtered water and electrolytes, were the inverse of those seen on cooling.

The experimental design adopted in these studies was unfortunately such as to encourage the development of mild renal ischaemia. Transient proteinuria and a fall in TmPAH occurred during the rewarming-bypass period. While these are almost certainly anoxic phenomena, it cannot be stated categorically that they are not representative of unknown, possibly harmful, effects of hypothermia.

RENAL EFFICIENCY:

The calculation of renal efficiency has, in the past, been based on the premise that "the function of the kidney is to produce urine". Yet cold-induced paresis of renal function causes a rise in urine production (where the rate of urine flow is expressed as a percentage of the glomerular filtration rate). It might then be stated, paradoxically, that is a function of the kidney not to produce urine. These considerations suggest that the calculations of renal efficiency be revised.

ACKNOWLEDGMENTS.

This Thesis embodies, in varying measure, the labours of a number of members of the staffs of the Departments of Surgery and Medicine, of the University of Cape Town.

My thanks are due to Prof. J.H. Louw, and Mr. C.N. Barnard, of the Department of Surgery, for their continued interest and support.

Professor L. Eales, head of the Renal-Metabolic Group of the Department of Medicine, gave of his interest and encouragement throughout the course of this work; he also generously allowed me free access to the facilities of the A18 chemical laboratory.

Dr. J. Terblanche, then Surgical Registrar to the Department of Experimental Surgery, performed most of the major surgery. Without his skill and patient co-operation under often trying circumstances, this Thesis could not have been written. His successor, Dr. P. Baker was also most helpful, as were the technical staff of the Experimental Surgery Laboratory, Messrs. C.C. Goosen, C.J. Lockett, and Mrs. V.M. Connell.

The photographs were prepared by Mr. I. O'Reilly, technical assistant to the Department of Medicine.

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APPENDIX A.

HYPOTHERMIC BUBBLE OXYGENATOR.

The desirability of combining hypothermia with extracorporeal circulation has been pointed out in Chapter 1. Use of the latter lends itself to the application of blood-stream cooling, a fusion of techniques first practised by Peirce and Polley (128) in 1953.

The apparatus used here, for the induction of profound hypothermia, follows these principles. The extracorporeal circulation was supplied by a slightly modified De Wall Helix-Reservoir Bubble Oxygenator (11, 46). Full particulars regarding local usage of this system have been published (10, 109). Two modifications were introduced (11) for the induction of hypothermia. Venous blood was drained directly into the mixing chamber, thus dispensing with a venous pump; and the helix reservoir was not immersed in a water bath, as the dogs temperature was to be controlled by means of a Bennington heat exchange unit in the arterial line.

The circuit is shown in the Figure 28 (taken from ref. 11) which is self-explanatory.

HYPOTHERMIC BUBBLE OXYGENATOR

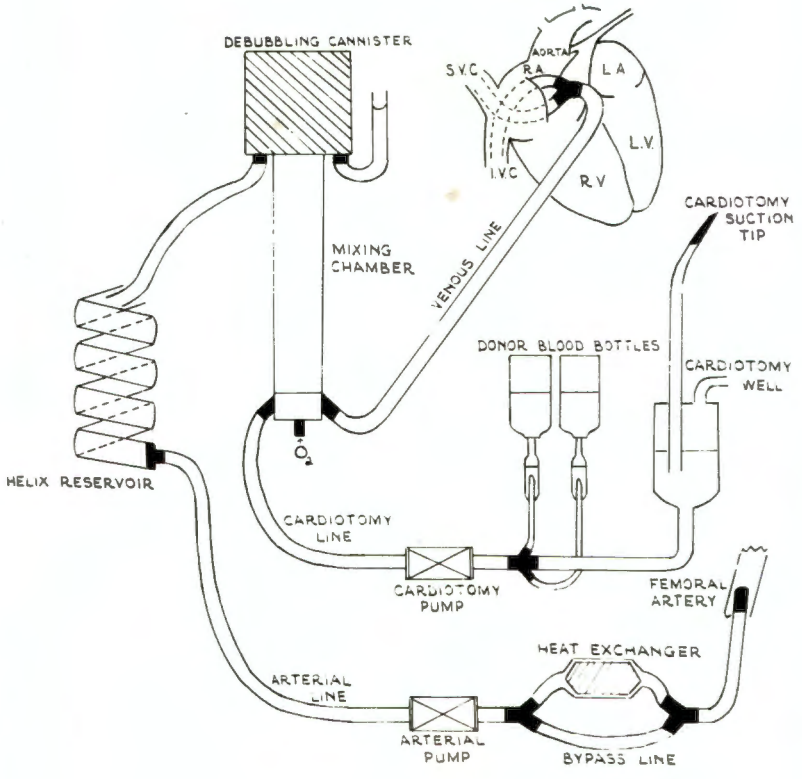


FIG. 28.

APPENDIX B.

OPERATIVE AND BYPASS PROCEDURE.

Healthy adult mongrel dogs, weighing between 15 and 21 kg., were anaesthetised with intravenous thipentone sodium, and after intubation, anaesthesia was maintained with nitrous oxide and oxygen. Polyethylene cannulae were inserted into the left femoral artery and vein, and connected to manometers for pressure recordings. One needle was inserted into a vein in one of the forelimbs and a second into a vein in the right hindlimb for intravenous infusions during the experiments.

In those dogs in which renal function was studied under normothermic conditions, the heart was exposed through a right thoracotomy. In the remainder, in which profound hypothermia was induced, the heart was approached by a left thoracotomy through the 4th interspace.

After systemic heparinisation with 1.5 mg. intravenous Heparin per kg. of bodyweight, the right common femoral artery was exposed and catheterised with a stainless steel catheter. The arterial line of the Helix reservoir bubble oxygenator (cf Appendix A) which had been previously assembled and primed with heparinised donor blood, was connected to the arterial catheter. Bennington heat exchange units[x]

x Supplied by Westdene Products (Pty) Ltd., Cape Town.

were incorporated in the arterial line when hypothermia was to be induced.

In the hypothermic dogs, the venous blood was drained by means of a single No. 32 Bardic catheter, introduced into the right atrium through the right atrial appendage; once hypothermic failure of the heart had occurred, a second catheter was introduced into the left atrium through the left atrial appendage, and the blood aspirated from the left heart was returned to the heart-lung machine in this manner. In the two control dogs, the venous blood was drained by means of catheters in the superior and inferior venae cavae.

Flow rates of 70 ml. per kg. bodyweight per minute were kept up throughout the perfusions of all animals, except in dog A, where extracorporeal circulation was stopped completely for 32 minutes during the cold phase. No cardiac surgery was performed during any of these experiments. Rectal and oesophageal temperatures were recorded in the dogs using the Electric Universal Thermometer Type TE-3, with lead types R-7 and OSG-1[xx]

At the end of the perfusion, when a normal heart beat has been established, bypass was discontinued. The heparin was neutralised with the required amount of Protamine sulphate, and blood deficit was replaced through

xx Manufactured by Elektrolaboratoriet, Copenhagen.

the arterial catheter using arterial and venous pressure as monitors.

The wounds were closed and a chest drain was attached to a suction apparatus. The dogs were then returned to a warm recovery cage. No special postoperative care, apart from overnight intravenous fluids and routine antibiotics, was adopted.

Urine collections were obtained from a catheter tied in one, or both (Dogs A and B) ureters. In the former, a ureter was exposed through a small flank incision and dissected out retro-peritoneally. The tip of the catheter was inserted into the renal pelvis and tied firmly into place. In dogs A and B, both ureters were catheterised via the bladder, after opening the latter by a midline, anterior abdominal, transperitoneal approach.

FIG. 29.

LEGEND TO FIG. 29:

The surgeons preparing a dog for cardiopulmonary bypass under hypothermia. The dog's forepaws are just visible, protruding from beneath the green drapes. The extracorporeal circulatory and cooling devices are on the right of the photograph. The pressure cylinder (right foreground) supplies oxygen to the mixing chamber, the vertical blood-filled tube seen surmounted by the debubbling cannister. The helix-reservoir stands between the oxygen cylinder anteriorly, and the sigmamotor pump posteriorly and inferiorly.
The technician responsible for the maintenance of this machinery is seen in the background.

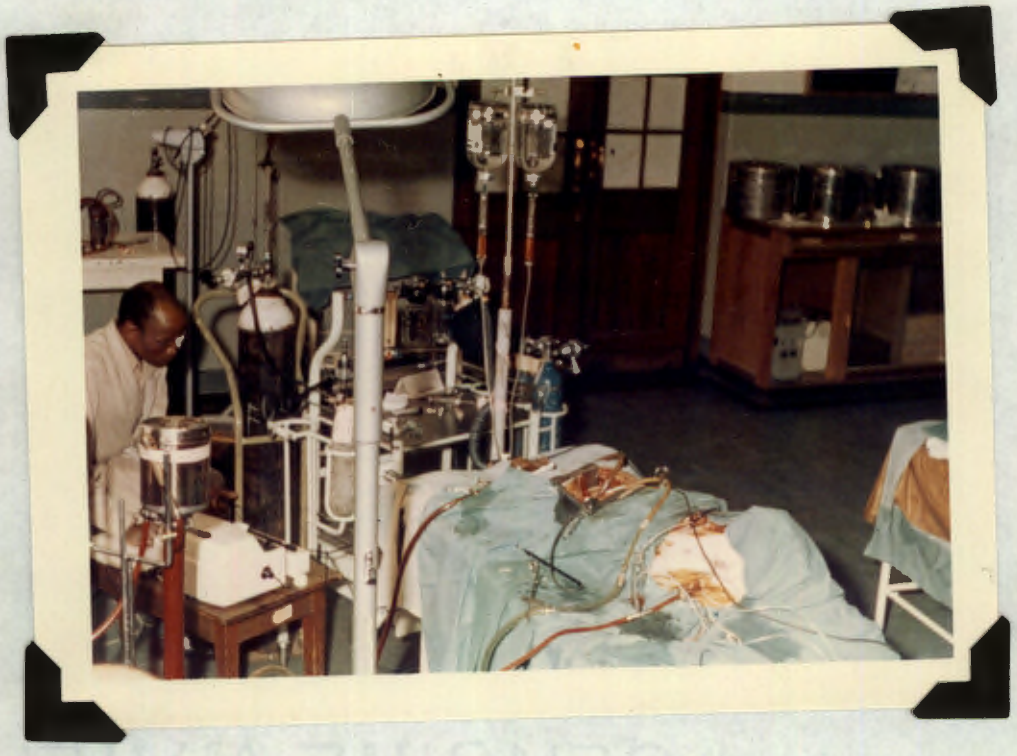


FIG. 30.

LEGEND TO FIG. 30:

LATER. The surgeons have finished their work, and an experiment on the renal effects of hypothermia is under way. The dog lies beneath the green drapes, with its feet towards the camera. The drip stand, bearing vacolitres of 10% dextrose-water, and of 'normal' saline containing creatinine and PAH, is seen at the head of the table. Behind it is the anaesthetists trolley. To the left, is the laboratory assistant responsible for the constant monitoring of oesophageal and rectal temperatures.

Three surgical wounds are visible. The most cephalic is the thoracotomy wound, from which the venous catheters emerge. The most caudal is for the insertion of the arterial catheter into the femoral artery. Between these, a ureteral catheter and thermistor probe cable emerge, from a left flank incision.

FIG. 31.

LEGEND TO FIG. 31.

A close-up view of the left flank incision. A polyethylene ureteral catheter leads to a urine collection bottle. The black cable running in the opposite direction is that of the thermistor probe, which in this particular experiment was plunged into the substance of the kidney. This no doubt lead to the trickle of blood seen in the proximal segment of the ureteral catheter. The thin threads lying on the dog's anterior abdominal wall are the ends of the ligature tying the catheter into the ureter.

APPENDIX C.

SOME FIGURES ON NORMAL RENAL FUNCTION IN THE DOG.

Values for GFR, RBF and TmPAH are usually related either to the body surface area, or to body weight. Houck (82), in a study of renal function in normal dogs, found almost no difference between the correlation coefficients for either of these methods. In his series of 75 dogs, the ratio of body weight (in kg) to surface area (in square metres) was 19.7. Following Smith (157) I have used this ratio where necessary to convert figures from one reference standard to the other; such conversions are here denoted by an asterisk.

SOURCE	GFR (ml./min)		CPAH (ml./min.)		TmPAH (mg./min.)		EXTRACTION RATIO of PAH
	per m^2 surface area	per kg body wt.	per m^2 surface area	per kg body wt.	per m^2 surface area	per kg body wt.	
Smith (158) 1956.	84±19.1	4.3±1.01	266±66	13.5±3.26	19.1±1.8	0.97±.09	0.917
Ashein et al (6) 1961	Mean:76 Range 42-102 S.D. 15	3.77 1.74-5.86 0.84	263 160-419 62	12.88 6.30-21.18 3.21	Big variations in TmPAH 1-55	.051-2.81	
Kubicek et al (97) 1953	~~88.4 ± 12.1~~ ~~8.65±13.7~~	~~* 4.49 ± .61~~ ~~4.39±.7~~	~~286 ± 505~~ ~~279±44.3~~	~~* 145 ± 2.56~~ ~~14.2~~			0.90
Kolberg (96) 1959	Cor: 55.9;S.D. 12.2 C_{IN} 64.9;S.D. 13.4	*2.83;S.D. .62 *3.29;S.D. .68	191.6 S.D:46	*9.75 S.D:2.3	16.2 S.D. 3.99	*0.83 S.D. .20	

The data given by Smith (158) may be regarded as composite figures based on the literature prior to 1951.

APPENDIX D.

METHODS OF CHEMICAL ESTIMATIONS.

OSMOLALITY: of serum and urine samples was determined by measurement of the depression of freezing point. This is based on the approximate relationship (ref.32).

$$\Delta t = K_f M$$

where Δt = depression of freezing point of solvent.

K_f = a proportionality constant, or 'cryoscopic constant'. This varies with different solvents.

M = molal concentration of solute.

The cryoscopic constant of water is 1.86, i.e. an aqueous solution containing 1000 mOsm/per kg. water will freeze at - 1.86°C.

A Fiske osmometer, calibrated with NaCl standards, was used, and was accurate to 2%.

The Na and K content of both serum and urine samples was determined by flame photometry. A Barclay flame photometer, which compares the emission of an 'internal standard' (lithium sulphate) to that of the unknown, was used. Lithium sulphate is added to the solution to be tested, and the response of one photoelectric cell to the emission of lithium is compared with the response of another photoelectric cell to the emission of Na or K. The accuracy of the method is of the order of 1%.

The inorganic phosphate, para-aminohippurate, and protein

concentrations of urine, and the inorganic phosphate and para-aminohippurate concentrations of serum, were estimated following the methods described by King and Wootton (92).

Inorganic Phosphate: content of both serum and urine samples was determined by the addition of ammonium molybdate to form a yellow phospho-molybdate. This was then reduced, by the further addition of ascorbic acid, to yield a blue colour, the intensity of which was compared to that of a standard inorganic phosphate solution, similarly treated, in a Klett Summerson photoelectric colorimeter.

Para-aminohippurate was estimated as for the determination of nonconjugated sulphanilamide in blood or urine. Sodium nitrite was added to deproteinised plasma, or diluted acidified urine, to diazotise the PAH; ammonium sulphamate was then added to destroy the excess sodium nitrite; and finally naphthylethylenediamine was coupled with the diazonium compound, to yield a pink solution. The intensity of this colour was then compared to that of a standard PAH solution, similarly treated, on a Klett-Summerson photoelectric colorimeter.

Urine Protein Content. Three volumes of 3% (W/V) sulpho-salicylic acid was added to one volume of clear urine.

Proteinuria when present, caused turbidity. This was then compared with that of a set of (ten) gelatin permanent standards. These range from 0 to 100 mg/100 ml.; this enabled one to determine the degree of proteinuria to the nearest 10 mg/100 ml. within this range.

Creatining was determined by the method of Folin and Wu. This is based on the Jaffe reaction i.e. creatinine, when mixed with an alkaline picrate solution, gives rise to a reddish brown colour.

After addition of diluted urine, or protein-free serum filtrate to the picric acid-sodium hydroxide mixture, the solution was allowed to stand at room temperature for just 10 minutes, whereafter the colour intensity was immediately compared to that of a standard solution, in a Klett-Summerson photoelectric colorimeter.

Both serum and urine samples in all our experiments contained large amounts of glucose. Glucose itself reacts with alkaline picric acid mixtures to produce a dark brown colour. To reduce this interfering effect, 3% NaOH was used in place of the more usual 10% NaOH, and the solutions were read precisely 10 minutes after preparation. No more than 5 to 8 solutions were read at a time, in order to avoid delay between the readings of the first and last samples.

Calcium concentrations of both serum and urine samples were estimated by the method of Kingsley and Robnett (94) This utilises a dye stuff, 'Corinth Ca', (or Disodium 1-hydroxy-4-chloro 2, 2 diazobenzene-1, 8-dihydroxynapthalene-3, 6 disulphonic acid) which changes colour, from violet to pink, in the presence of calcium. While the error, in my hands, was as high as 10%, the method had the great advantage of utilising only 0.2 ml. of the solution to be tested.

Magnesium was determined by flame spectrophotometry, using the Zeiss PMQ 11 spectrophotometer, with a single monochromator, to measure Mg emission at 285.2 mμ.

Sodium emits weakly at 285.3 mμ, and causes some interference with the emission of Mg. As the two emissions are simply additive, the former can readily be corrected for, once the Na concentration of the solution under test is known.

Serum was prepared for Mg determination by preliminary deproteinisation, and the addition of acetone (cf Van Fossan D., Baird E., Tekell G., (1959). "A simplified flame spectrophotometric method for estimation of magnesium in serum". Am. J. Clin. Path. 31:368).

Urine was prepared simply by diluting, as necessary, with distilled water. The minimal amounts of proteinuria

encountered in this study were insufficient to interfere with the emission of Mg.

Serial dilutions, replicates, and recoveries performed on random serum and urine samples indicate that the method has an error of about 5%. Details are to be published elsewhere.

APPENDIX E.

ACTIVATION ENERGY.

To the clinician, the concept of Activation Energy is still a novelty. Only the barest outline of the theory is given here; fuller treatment can be found in the texts cited below.

Molecular energy, and therefore the number of intermolecular collisons, varies directly with the absolute temperature. At room temperature (e.g. $27^{o}C$), a rise of 10^{o} represents only $\frac{10}{300}$, or a 3% increase in molecular energy. The number of intermolecular collisions, or speed of reaction between substances, may therefore be expected to increase proportionately. While this has been found to be true of a number of purely physical processes, such as diffusion, or radio-active decay rates, chemical and biological reaction velocities often show considerably greater increases. The concept of Activation Energy was first introduced by Arrhenius (1889) in an attempt to explain these apparent anomalies.

Arrhenius postulated that to participate in a chemical reaction, molecules needed to have a higher energy content than usual; these he called 'reactive molecules'. In any

substance, the reactive molecules existed in equilibrium with the inactive, low energy molecules. He introduced the equation

$$\frac{d(\ln k')}{dT} = \frac{E}{RT} \qquad \cdots (1)$$

where k' = rate of the chemical process at absolute temperature T, R = gas constant, and E was the amount of energy needed to convert normal inactive molecules into reactive ones. The larger the value of E, the more rapidly the reaction rate increased for a given rise in temperature.

On intergration, equation 1 becomes:

$$\log k = \frac{E}{2.3R}\left(\frac{1}{T}\right) \qquad \cdots (2)$$

From this, one can derive:

$$\log\left(\frac{k1}{k2}\right) = \frac{E\ (T_1 - T_2)}{2.3R(T_1\ T_2)} \qquad \cdots (3)$$

where k_1 and k_2 are the reaction rates at absolute temperatures T_1 and T_2 respectively. Equation (3) can be used to derive E directly from experimentally obtained data. Alternatively, graphic plotting of the logarithm of the reaction rate against the reciprocal of the absolute temperature, at different temperatures, yields a line, the slope of which equals $\frac{E}{2.3R}$ (equation 2).

E, the Activation Energy, is expressed as calories/mol./ degree. In biological systems, where the nature and number

of the reacting molecules are usually unknown, or ill-defined, E is usually referred to as the Apparent Activation Energy, and is expressed as calories/degree.

As far as it went, the Arrhenius equation was of use to biologists only as a means of expressing variations in rate of various processes, with changing temperature. The significance of the value of E, in absolute terms, remained an enigma.

In 1935 Eyring modified and extended the above, and the so-called Absolute Reaction Rate Theory was born. According to this view, when molecules a and bc react to form ab + c, an intermediate complex, a-b-c, is formed. That is,

$$a + bc \rightleftharpoons a\text{-}b\text{-}c \longrightarrow ab + c$$

The formation of the complex a-b-c requires energy, and it is this energy barrier that prevents the reaction $a + bc \longrightarrow ab + c$ from proceeding spontaneously. The complex a - b - c is referred to as the "Activated Complex', in place of the more nebulous 'Reactive Molecules' of Arrhenius.

The concepts of classical chemical thermodynamics were applied to the formation of the activated complex, and starting with the basic premise that:

$$\Delta F^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger}$$

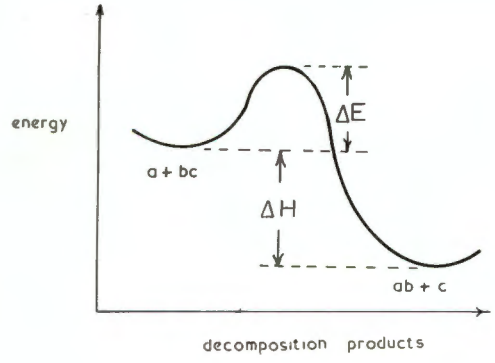
where $F^{\ddagger}$, $H^{\ddagger}$ and $S^{\ddagger}$ are the Free Energy, Enthalpy and Entropy of activation respectively.

it was shown that the Arrhenius factor, E, was numerically equal to $\Delta H^{\ddagger} + RT$. As R, the gas constant, is 1.99 cals./degree/mol. and as at a room temperature of 27°C, T equals 300° Absolute, the term RT approximates to 600 cals/degree/mol., for most biological systems. H , on the other hand, is usually of the order of several thousand calories/degree/mol., and as biological experimentation seldom yields data of great quantitative accuracy, the term RT is usually ignored. The Arrhenius factor, E, can therefore be equated with the more meaningful factor, $\Delta H^{\ddagger}$.

It was further shown that the speed of the reaction a + bc → ab + c, depended on the Free Energy of Activation i.e. $\Delta F^{\ddagger}$. Usually, this varies concomitantly with $\Delta H^{\ddagger}$.

Biological reactions usually undergo rate changes similar to those shown in figure 32 (where the logarithm of the rate is plotted against the reciprocal of the absolute temperature). As the temperature rises, the reaction speed increases, to reach a plateau at the optimum temperature. Above this temperature, the reaction rate declines. The

modern concept of this phenomenon is that it represents the interplay of at least two opposing chemical reactions. These are (1) the acceleration of the chemical reaction under observation, with rising temperature (2) the progressive denaturation of the participating enzymes, at temperatures above the optimum. Each of these reactions is susceptible to analysis in terms of the Absolute Reaction Rate Theory, and given the experimental data (of reaction rates above, below and at, the optimum temperature) it is possible to derive the values of $\Delta F^{\ddagger}$, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$. These values, in turn, enable the biophysicist to gain new insight into protein interactions and deformations occurring during the chemical process under observation.



$$\frac{d \ln k}{dT} = \frac{\Delta E}{RT^2}$$

ie. $$\log k = \frac{\Delta E}{2{\cdot}3R}\left(\frac{1}{T}\right)$$

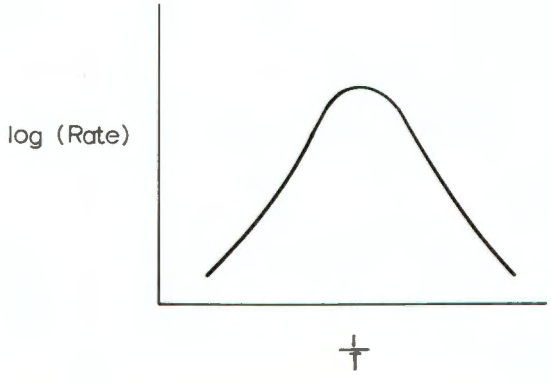


FIG. 32.

LEGEND TO FIG. 32:

E represents the additional energy needed for the formation of the activated complex a-b-c, from the molecules a and bc. It corresponds to the Activation Energy, E (Arrhenius), or to $\Delta H^{\ddagger} + RT$ (Eyring).

H is not referred to in this Thesis. It represents the difference in Heat Content, or Enthalpy, between systems a + bc, and ab + c, and is that quantity usually measured in biological work, by the bomb calorimeter. It is included here to help point out the difference between it and E.

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