ALBUMIN METABOLISM
FOLLOWING
PARTIAL HEPATECTOMY
IN THE RAT

by

ELWYN ALLDEN LLOYD
M.A., B.M. (Oxon), F.C.P. (S.A.)

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TO SHERRY
WITH MY LOVE
NORMAL ADULT RAT LIVER

IMMEDIATELY AFTER PARTIAL HEPATECTOMY

Caudate lobe; anterior and posterior segments of right lateral lobe. Note last named crossing the upper pole of the right kidney.
8 HOURS AFTER PARTIAL HEPATECTOMY

Note fatty infiltration

10 DAYS AFTER PARTIAL HEPATECTOMY

Note hypertrophy of the lobes; the posterior segment of the right lateral lobe now extending well below the lower pole of the right kidney.
ACKNOWLEDGEMENTS

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INDEX

REVIEW OF LIVER CELL PROLIFERATION
INTRODUCTION
PRINCIPLES OF METHODOLOGY
MATERIALS AND METHODS
RESULTS
DISCUSSION
SUMMARY
APPENDIX

A. Liver Perfusion
B. Partial hepatectomy
C. Determination of Plasma Albumin Concentration
D. Amino Acids
E. Measurement of Albumin Synthesis
F. Determination of Urea Concentration (Nessler's method)
G. Preparation of Albumin by Polyethylene-Glycol method
H. Iodination
I. Determination of Urea Concentration (Diacetyl Monoxime Method)
J. Measurement of Albumin Catabolism
K. Statistical methods

REFERENCES
REVIEW OF LIVER CELL PROLIFERATION
REVIEW OF LIVER CELL PROLIFERATION

The latent capacity of the liver for growth, which is unmatched by any other mammalian organ, has long been and still is a source of fascination. In spite of the fact that mature liver cells are long lived - in rats and mice they may even survive for the adult life of the animal - the simple expedient of partial hepatectomy sets into motion a burst of astonishingly rapid proliferation. In the normal rat and mouse, one mitosis is seen in about 10,000 to 20,000 hepatocytes, just sufficient to allow for normal body growth. Following partial hepatectomy, however, the rate of cell division is nearly once in every 24 hours, a rate which is as rapid as that seen in many embryonic and cultured cells and exceeds that in most neoplastic cells (Brues et al, 1936; Fabrikant, 1967). In addition to this, the growth is finely controlled, tapering off and ceasing when restoration of the original cell mass is complete.

The proliferative capacity of the liver is well illustrated in the rat by the almost complete restoration of its mass after repeated resection, even when a partial hepatectomy is performed every month for a year (Ingle and Baker, 1957; Simpson and Finckh, 1963). Liver mass is restored with extraordinary rapidity, the residual lobes in normal adult animals nearly doubling in size in 48 hours and approaching the original liver weight in seven days (Brues et al, 1936; Bucher and Malt, 1971a).
Some discussion has ensued regarding the term 'regeneration' as it is used in the context 'liver regeneration'. Fishback wrote in 1929: "The real meaning of 'regeneration' is restoration of tissue or part of an organ at the site of its removal, and the term implies compensation, either structural, as of a part, or physiologic, as of a function. In the restoration of hepatic tissue after partial hepatectomy, the replacement does not occur at the stump from which the lobes are removed, but within the remaining lobes, so that 'restoration' or 'restitution' might describe the process more accurately".

Fishback was supported by Higgins and Anderson (1931): "In the case of the mammalian liver one is dealing with a response in growth considerably different from that which ensues on the removal of the tadpole's tail. Following partial removal of the liver, proliferation of cells does not ensue at the level of the cut; new lobes do not develop to take the place of those removed. There is, on the other hand, hyperplasia of the remaining lobes. There are no centres of cell proliferation, but generalized mitotic activity, coupled with cellular migration and differentiation, results in the formation of new lobes, and the pre-operative weight of the liver is thus rapidly restored. Regeneration as employed biologically would involve, it seems to us, the replacement of a removed part, with rearrangement or reorganization of the old part. This term could well be applied to the recovery of the hepatic lobule from chemical injury as, for example,
chloroform or carbon tetrachloride poisoning, in which there is not only reorganization of portions of the old lobule, but new cellular proliferation as well. Bucher and Malt (1971b) added further support: "Regeneration of the liver is more properly described as a compensatory hyperplasia which involves growth of residual lobes rather than regrowth of those that were excised". These authors appear to be supported by the Shorter Oxford English Dictionary which defines the verb 'regenerate' thus: "\ldots\ldots\ldots\ldots\ldots(2)\ldots Path. To reproduce, form afresh (some part of the body)". The author has accordingly, in keeping with current terminology, used the term 'liver cell proliferation', which accurately describes the phenomenon.

It is the early period of cellular growth and division that is the time of greatest interest, when metabolic activity centres around synthesis of new hepatic cells, before moving toward compensation of impaired liver function. In the rat the period of maximal liver cell proliferation is at about 22 hours after hepectectomy (Grisham 1962). In man it is estimated, by means of scintillation scanning studies using various radioactive substances, that the hepatic mass is fully restored in about six months (McDermott et al, 1963).

At the microscopic level, changes occur first in the parenchymal cells, which constitute 90-95% of the total hepatic cellular volume, but only 60-65% of the cell population, there being infiltration with lipid and loss of glycogen within a few hours. The cells,
nuclei and nucleoli enlarge, smooth endoplasmic reticulum increases, lysosomes may become more prominent, and some autophagocytosis is observed. Deoxyribonucleic acid (DNA) synthesis is first observed 16 - 18 hours after partial hepatectomy and occurs first in cells at the periphery of the lobule, later spreading toward the centre, reaching peak activity at 20 - 24 hours, then declining. Mitosis follows the same pattern 6 - 8 hours later. In proliferation, compared to developmental growth, the cell cycle is shortened, especially in the G₁ phase, after an initial lag (Bucher and Malt, 1971c), (Fig. 1). After partial hepatectomy (or treatment with growth hormone) polyamine synthesis is stimulated; the increase in ornithine decarboxylase (which mediates the first step in biogenesis of the polyamines spermine and spermidine) activity being manyfold within as short a space of time as 15 minutes (personal observation). The half-life of ornithine decarboxylase is 11 minutes in both normal and partially hepatectomized livers and is the shortest on record for any enzyme, its nearest rival being 6 - 7 times longer (Russel and Snyder, 1969).

The idea that the remaining mass of the liver is quickly restored after loss of a portion comes down from antiquity. Fishback (1929) suggested that the phenomenon was first revealed in the myth concerning Prometheus, he having to pay cruelly for his trickery and thefts. At the command of Zeus, Prometheus was bound with indestructible chains to one of the crests of Mount Caucasus. There
Figure 1. The cell life cycle.

S, period of DNA synthesis; D or M, period of mitosis & division; G₁ & G₂, intervals (gaps) between these sharply defined nuclear events.
"an eagle with outstretched wings, sent by Zeus, fed upon his immortal liver; as much as the winged monster devoured, during the day, that much grew again during the night".

An indication of the degree of interest which has long been shown in this subject is given by Fishback when, as long ago as 1929, he described the literature on liver cell proliferation as "exceedingly comprehensive". However, the mechanism by which this liver cell proliferation is initiated and controlled remains tantalizingly obscure, although two distinct sources of the stimulus have been invoked; namely, a specific humoral agent, the concentration of which changes following partial hepatectomy and secondly, the haemodynamic or metabolic load imposed on the remnant as a result of resection.

While the intensive search for the clue to the control of liver cell proliferation is in part academic, its discovery has important clinical considerations: during the period 1963-1971, a total of 492 patients with the presumed diagnosis of acute hepatitis were treated by the Liver Research Group of the University of Cape Town. Of these, 119 patients (24.2%) had evidence of hepatic encephalopathy. Using a classification of hepatic coma adapted from Adams and Foley (1949), 51 patients were found to be in grade II or III coma while 68 patients with massive hepatic necrosis had grade IV coma. The survival rate of patients in deep hepatic coma without special treatment is under 10% (Trey and Davidson, 1970). While viral hepatitis accounted
for the majority of cases, the Cape Town series included a few patients with halothane hepatitis and drug induced hepatitis.

It has been suggested that the key to recovery from massive hepatic necrosis lies in liver cell proliferation (Saunders et al., 1972; Bucher 1978). Further, studies of this phenomenon of liver cell proliferation are relevant to neoplasia, especially with regard to the concept that in the adult stage the factors controlling cell proliferation may be switched 'on' and 'off' but in cancer they appear to be permanently 'on' (Potter, 1969).
INTRODUCTION
INTRODUCTION

While restoration of hepatic tissue by cellular growth and division is of clinical interest so, too, are the consequences of the inevitable impairment of hepatic function which must accompany reduction in hepatic mass - be this due to infection, destruction by toxins or partial surgical excision for trauma or neoplasia. Since partial hepatectomy brings about that rapid liver cell proliferation which is peculiar to the liver, any studies following partial hepatectomy are inextricably concerned with the phenomenon of liver cell proliferation.

One of the striking problems following partial hepatectomy in man is a fall in the plasma albumin which occurs in the immediate post operative period. Recent work reviewed here indicates that albumin metabolism may be influenced in vitro and in vivo by "hyperalimentation" with amino acids and other manoeuvres. It was accordingly decided to study albumin metabolism after partial hepatectomy and to evaluate the effects of supplementary amino acids and hydrocortisone on albumin synthesis in this situation.

Albumin metabolism has been extensively studied and reviewed (Tavill, 1972; Rothschild, 1973; Schreiber 1978) and it is not the author's intention that it be further reviewed here.

A great deal of information regarding albumin synthesis is available - it is produced in the liver and within the hepatocyte there exists a complex mechanism geared for the synthesis of export proteins.
This system consists of a strand of messenger ribonucleic acid to which is attached two ribosomal subunits (Blobel and Potter, 1967; Sabatini et al, 1966; Falvey and Staehelin, 1970; Weignand et al, 1971). The larger of these subunits is attached to the microtubular structure, the endoplasmic reticulum. As the albumin molecule is synthesized, it is inserted through the centre of the larger ribosomal sub-unit into the cisternal space of the endoplasmic reticulum and then, traversing both the smooth reticulum and the Golgi apparatus, is extruded in some fashion directly into hepatic plasma (Peters et al, 1971). In vivo, newly synthesized albumin is deposited directly into hepatic plasma and hepatic lymph plays an insignificant role in the delivery of albumin to the systemic circulation (Woolley and Courtice, 1962; Smallwood et al, 1968).

While it is well known that the plasma albumin level falls following partial heptectomy (Chanutin et al, 1938; Islami et al, 1956; Pack and Molander, 1960; McDermott et al, 1963; Monaco et al, 1964; McDermott and Ottinger, 1966; Almersjö and Bengmark, 1969; Aronsen et al, 1969; Dillard, 1969; Flatmark et al, 1973) and after major surgery (Hoye et al, 1972) the relative contribution of alterations in dietary intake, albumin synthesis rate or albumin catabolic rate have not been delineated. There is little literature available concerning albumin synthesis rates following partial heptectomy and that which is available is conflicting (vide infra).
As early as 1938, Chanutin et al reported a fall in plasma albumin following partial hepatectomy in rats. In 1951, Miller et al published a paper establishing the liver as the sole source of albumin synthesis: a fall in plasma albumin is hence the anticipated consequence of partial hepatectomy, which removes a good portion (2/3rds) of the albumin synthetic machinery. Using liver slices, Guidotti et al (1959) showed no significant change, in terms of liver weight, in albumin synthesis rates following partial hepatectomy, although observing an increase in 'albumin turnover'. He suggested the fall in plasma albumin was due to increased utilization of plasma albumin in hepatic tissue restoration. This was confirmed (in terms of liver weight) by Braun et al (1962) using incorporation of $^{14}$C-amino acids into albumin in vivo and in vitro (tissue slices), by Rosenoer et al (1970) using the isolated perfused rat liver and by Schreiber et al (1971) using the $^{14}$C-leucine technique in vivo.

In 1966, however, Mutschler and Gordon, using isolated rat liver perfusions and the $^{14}$C-leucine incorporation technique, showed a diminished synthesis of albumin during regeneration. These results were later confirmed, in terms of liver weight, by Murray-Lyon and Muller-Eberhard (1973) in vivo studies.

Yet more conflicting results were produced by Majumdar et al (1967), using the $^{14}$C-leucine incorporation technique in tissue slices, who demonstrated that partial hepatectomy and acute distress (produced by intra-peritoneal injection of Celite) caused an increase in the albumin synthesis rate, in terms of liver weight.
As discussed later in principles of methodology, the techniques used by some of the above workers for measuring albumin synthesis are open to criticism.

The factors regulating degradation of plasma albumin are not well understood: moreover, degradative mechanisms and sites are unknown. While the factors controlling albumin catabolism are not altogether clear, it has been shown to be influenced by a number of factors. Plasmaphoresis (Hoffenberg et al, 1966), protein calorie malnutrition (Kirsch et al, 1968a and Kelman et al, 1972a) and protein deprivation (Hoffenberg et al, 1970) have all been shown to cause a reduction in the albumin catabolic rate. It is interesting to note that in protein calorie malnutrition the fall in the albumin catabolic rate follows a fall in the plasma albumin pool which in turn is secondary to a reduced albumin synthesis rate.

Albumin is not a suicidal protein and the degradative mechanism handles old and new molecules alike. Thus, wherever the degradative system is located, the cell membrane must find an affinity for this albumin molecule regardless of its life span. It is conceivable that this liver produced protein is somehow considered foreign by this degradative mechanism and that the degrading system is saturated with albumin molecules at all times. (Rothschild, 1973).

Many tissues have been implicated and studied as potential sites for this degradative mechanism. The kidney has been shown not to play a significant role in albumin degradation in the absence of renal disease, but may play an active role in disease (Jensen et al, 1967; Marsh and
Drabkin, 1958; Bourdeau et al, 1972; Mogielnicki et al, 1971). In vitro studies indicate that the liver does not account for more than 10 - 14% of the total albumin catabolic rate (Cohen and Gordon, 1958). The intestinal tract is another organ which has been found to play a significant role in albumin degradation in disease but not to be of importance in the normal state (Jeejeebhoy et al, 1969; Kerr et al, 1967). Loss through the normal intestinal tract again probably accounts for less than one tenth of the total albumin degradation.

While albumin catabolism following major surgery (Hoye et al, 1972) burns (Birke et al, 1959/60) and protein calorie deprivation (Kelman et al, 1972a; Kirsch et al, 1968a; Hoffenberg et al, 1970) has been extensively investigated, studies of the degradation of albumin following hepatic resection are limited and the results are conflicting (Almersjö and Bengmark, 1969; Scornik, 1972). The former workers, using $^{131}$I and $^{125}$I labelled albumin, in humans subjected to 20 - 80% hepatic resections observed a rise in albumin catabolism no different to that occurring after major surgery. Scornik, on the other hand, using mice and L-arginine (guanido - $^{14}$C) found a dramatic reduction in the rate of protein degradation following partial heptectomy.

Of the many factors influencing albumin synthesis, the most important appears to be nutrition and more specifically the supply of amino acids. Withholding food from animals causes a reduction in albumin synthesis of the order of 50 per cent which occurs within 24 hours.

Using the isolated perfused rat liver the role of amino acids in the regulation of albumin synthesis has been studied extensively. Individual amino acids, as well as mixtures of amino acids, have been shown to enhance albumin synthesis in malnutrition (Kirsch et al, 1969; Rothschild et al, 1969; Kelman et al, 1972b; Lundholm et al, 1977) and after exposure of the liver to alcohol (Rothschild et al, 1971; Kirsch et al, 1973; Jeejeebhoy et al, 1972).

The effect of cortisone on albumin synthesis and catabolism has been investigated (Grossman et al, 1960; Schauder and Buck, 1971) and has been shown to increase albumin synthesis per se (Bancroft et al, 1969).

It will be observed that some of the literature quoted in this introduction is ten years old and that the laboratory work on which this thesis is based was carried out in 1972 and 1973. Due to an unexpected increase in clinical responsibilities the author has been unable to publish this thesis earlier but every effort has been made to update the literature and it transpires that while the results published in this thesis have stimulated further research (see Discussion) the work has not been duplicated as far as can be established by a thorough examination of the English language literature.
The author could find no studies in the English literature concerning the effects of amino acids or cortisone (or related compounds) on the albumin synthesis rate following partial hepatectomy. In the work reported here the author set out to investigate five aspects of albumin metabolism following partial hepatectomy in the rat.

1. The plasma albumin levels following partial hepatectomy.
2. The effect of partial hepatectomy on the albumin synthesis rate.
3. The effect of supplementary amino acids on the albumin synthesis rate following partial hepatectomy.
4. The effect of hydrocortisone on the albumin synthesis rate following partial hepatectomy.
5. The effect of partial hepatectomy on the albumin catabolic rate.
PRINCIPLES OF METHODOLOGY
1. **Albumin Synthesis**

The use of radioactive isotopes in research is now taken for granted. Prior to their advent nitrogen balance studies were carried out, when albumin synthesis was derived from catabolic studies of subjects in nutritional equilibrium when synthesis was presumed to equal catabolism. This had many disadvantages, not least of which was its dependence on steady state conditions.

The method by which radioactive isotopes are employed for measurement of albumin synthesis rates is based on the precursor-product principle. Labelled amino acids are used and synthesis determined from the ratio of protein-bound radioactivity relative to the labelled amino acid at the site of protein synthesis. The main source of inaccuracy of this method is the difficulty of deriving precursor amino acid radioactivity at the site of protein synthesis since this is dependent on so many variables, viz: rate of incorporation of labelled amino acids into the protein, which in turn is dependent on the ratio of labelled amino acids to the total concentration of that amino acid; this depends again on the dose of labelled amino acids administered, blood flow to the organ, transport across cell membrane, intracellular compartmentation, synthesis and degradation of amino acids, catabolism of protein and supply of unlabelled amino acids (Fig. 2).
Figure 2. FACTORS INFLUENCING THE PRECURSOR AMINO ACID SPECIFIC ACTIVITY
The need for an accurate extra-cellular indicator of intra-cellular precursor activity stimulated the work of Delluva and Wilson (1946), who demonstrated that $^{14}\text{C}$ carbonate combines with endogenously produced ammonia and ornithine in the liver to form arginine labelled only in the guanidine carbon atom. Some of the arginine is then used in the synthesis of hepatic proteins including albumin while the guanidine carbon of other arginine molecules is removed by arginase resulting in the production of $(^{14}\text{C})$ urea. (Fig. 3). Swick (1958) suggested that urea released from the liver could be used as an indicator of the specific activity of the guanidine carbon of intra-cellular arginine. This work led to the development of the technique known as the $(^{14}\text{C})$ carbonate technique for liver-produced plasma protein synthesis, the principles of which were defined independently by Reeve et al (1963) and McFarlane (1963a).

The method has been mathematically defined by Reeve et al (1963) and Reeve (1963) and in a series of papers a number of workers (McFarlane 1963a & b; McFarlane et al, 1965; Regoezzi et al, 1965; Rosenoer, 1967) have discussed the theoretical basis for the techniques and the various assumptions that need to be made in applying it, and have justified the method by comparing albumin synthesis rate derived from $^{131}\text{I}$ iodine-albumin studies in steady state animals with albumin synthesis rate from the $(^{14}\text{C})$ carbonate technique.
Figure 3. THE KREBS-HENSELETT CYCLE. The labelled carbon atom is marked by an asterisk.
The formula used by McFarlane (1963a) and by Reeve et al (1963) was derived as follows: The radio-activity appearing in a protein, and in any other direct product of 6-$^{14}$C-arginine, in a given time, is a function of the integral of the precursor arginine specific activity curve over the interval in question. Therefore, the radioactivity of the guanidine-carbon appearing in protein and urea in a given time will bear the same ratio as the respective masses of guanidine-carbon synthesized. That is to say: in two products arising from the same precursor the ratio of the activities of the products is equal to the ratio of the rates of incorporation of the precursor into the products:

<table>
<thead>
<tr>
<th>Synthesis rate of albumin guanidine carbon</th>
<th>Total activity in albumin guanidine carbon at time $t$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthesis rate of urea carbon</td>
<td>Total activity in urea carbon at time $t$</td>
</tr>
</tbody>
</table>

...... Equation 1.

The system is presented in simplified form in figure 4. The common precursor pool consists of units each emitting 10 counts per minute. For each molecule of arginine in albumin or of urea synthesized, one unit of precursor is depicted as being utilized.

Thus:

<table>
<thead>
<tr>
<th>Mass of albumin</th>
<th>radioactivity of albumin i.e. 50</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass of urea</td>
<td>radioactivity of urea</td>
<td>20</td>
</tr>
</tbody>
</table>

...... Equation 2.
Precursor pool (in cpm)

5 molecules of arginine in albumin

2 molecules of urea

Figure 4. Principle of the carbonate method.
If the total radioactivity incorporated into albumin and urea and absolute rate of urea synthesis are known, the absolute rate of albumin synthesis can be calculated.

**In vivo study**

A model of the system is shown in Figure 5. For practical purposes urea is synthesized exclusively in the liver (Bollman et al, 1924; Kornberg et al, 1952; Tavill et al, 1968). The mass of urea produced cannot be measured simply from the product of the fractional synthesis rate of urea and the urea pool as inaccuracies exist in the measurement of the size of the urea body pool (Regoezzi et al, 1965). In addition, the assumption that the urea is present throughout the body water at the same concentration as in the plasma may be invalid. After McFarlane et al (1965) equation 1 is modified by dividing both sides by the sizes of the urea carbon and guanidino carbon pools:

\[
\frac{FSR \text{ albumin}}{FSR \text{ urea}} = \frac{SA^* \text{ albumin at time}_t}{SA \text{ urea at time}_t}
\]

..... Equation 3.

(where \( FSR = \text{fractional synthesis rate} \)).

OR:

\[
FSR \text{ albumin} = \frac{FSR \text{ urea} \times SA^* \text{ albumin time}_t}{SA \text{ urea at time}_t}
\]

..... Equation 4.

* S.A. = Specific activity
EXTRAHEPATIC
ARGININE

PLASMA
ARGININE

LIVER
ARGININE

GUT UREA

UREA POOL

UREA
DEGRADATION

URINARY
UREA

ALBUMIN
CATABOLISM

PLASMA
ALBUMIN

EXTRAVASCULAR
ALBUMIN

DIET

Figure 5. The arginine, albumin & urea pools of the carbonate method in vivo.
At $t_0$ albumin SA = Specific Activity of the guanidine carbon of arginine in albumin at zero time.

The absolute synthesis rate of albumin (mg/24 hrs.) formula:
Albumin synthesis rate = FSR albumin x intravascular albumin pool

..... Equation 5.

In vivo, due to the losses of urea that occur during measurement (see Fig. 5), a hypothetical maximum value for ($^{14}$C) urea specific activity has to be obtained. This is done by extrapolating the curve of plasma urea specific activity to zero time. Similarly the hypothetical specific radioactivity of albumin was obtained by extrapolation to zero time, correcting the 6 hour value by a factor derived from the distribution and catabolism of the ($^{125}$I) albumin injected at the same time at $^{14}$C Carbonate.

In vitro (perfusion) study

The system is assumed closed and is depicted in Figure 6. Equation 1 can be modified.

Mass of albumin synthesized in time $t = $

Radioactivity incorporated into guanidine carbon of arginine in albumin $t$ x Mass of urea synthesised in $t$

Radioactivity incorporated into urea $t$.

..... Equation 6.

Previous workers in this laboratory have used this method extensively. Hoffenberg (1968) (in in vivo experiments) and
Figure 6. The arginine, albumin & urea pools of the carbonate method in the perfusion system (in vitro)
Kirsch (1968) have carried out further experiments validating the in vivo technique by comparing albumin synthesis rates derived from the \(^{14}\text{C}\) carbonate technique with those from albumin catabolic rates obtained using \(^{131}\text{I}\) Iodine-albumin in steady state animals.

2. **Albumin Catabolism**

Iodine labelled albumin was introduced in the 1950's in the measurement of albumin catabolism. When the \(^{131}\text{I}\) albumin molecule is degraded, the iodine attached to tyrosine is released. The behaviour of both the \(^{131}\text{I}\) iodide and the \(^{131}\text{I}\) albumin has been used as an index of albumin catabolism. Cohen et al (1956) and Campbell et al (1956) have shown that \(^{131}\text{I}\) is liberated only during reactions involving rupture of peptide bonds and the former worker excluded re-utilization of \(^{131}\text{I}\) iodide (in rats whose thyroid glands were blocked by prior administration of oral iodine), by failing to detect plasma protein labelling and by almost completely recovering the label in the urine within two days after feeding animals \(^{131}\text{I}\) proteins.

After intravenous injection of \(^{131}\text{I}\) albumin there is rapid distribution throughout the intravenous compartment, so that the degree of dilution 10 minutes after administration provides an index of the plasma volume. Distribution between the intra- and extracellular spaces takes place during the ensuing two to three days after which the semi-log plot of the plasma radio-activity against time is linear. (Fig. 7).
Figure 7. Whole-body (WBA), extravascular (EVA), & intravascular (IVA), activities after intravenous injection of labelled albumin. $T_E = \text{Equilibrium time.}$
If there are no losses in the urine, gut or other sites the albumin catabolic rates may be calculated from this slope (Sterling, 1951). However, catabolic rates measured in this way are underestimated as there is a bidirectional extra-intravascular movement of labelled albumin (Fig. 8). As the plasma ($^{131}$I) albumin starts to fall it will receive ($^{131}$I) albumin from the extravascular space. For this technique to be accurate there must be no net transfer of labelled protein from the extra- to the intravascular space - so called "equilibrium time". It has been proposed (Campbell et al. 1956) that at equilibrium time a tangent to the plasma ($^{131}$I) albumin semi-log plot against time (Fig. 7) would provide a measure of the albumin catabolic rate as a fraction of the plasma protein pool. The Y intercept of this line would provide the ratio of intra- to extravascular pool size. However, it has been found that the extravascular radioactivity required shows a broad plateau and the maximum point of the curve is difficult to define (Kelman, 1971).

Labelled iodide, released by catabolism is distributed throughout the body water, excreted in the urine, re-utilized by the thyroid gland and secreted and re-absorbed by the gut (Fig. 8). Thyroid uptake can be blocked by oral administration of stable iodine. Gut secretion can be ignored since iodide is almost completely re-absorbed. Zizza et al (1959), Lewaller et al (1959) and Mcfarlane (1963c) have established that the majority of non-protein-bound radio-activity is free iodide. The total iodide released from the labelled albumin, expressed as a fraction of the mean albumin-bound activity in the plasma, integrated over the corresponding 24-hour interval, is used
Figure 8. THE POTENTIAL PATHWAYS OF IODOALBUMIN
as a measure of albumin catabolism (Berson et al, 1953; Berson and Yalow, 1954). While accurate estimates of catabolic rates of plasma proteins depend on steady state conditions, the method used by the author has been widely accepted as valid even in the unsteady state (Mathews, 1961; Franks, 1963).

Activity in urine

\[\text{Activity in urine} = \frac{\text{Mass of albumin catabolised}}{\text{Plasma albumin SA}}\]

Accurate urine collection when using rats as experimental models is difficult. In the catabolic studies carried out by the author, therefore, urinary loss of radioactivity was not measured directly, but was derived from whole body counts. Experimental work in this laboratory carried out by Hoffenberg (1968) and Kirsch (1968) showed excellent correlation between this method and direct measurement of urinary \(^{131}\text{I}\) loss. Faecal loss does not contribute materially to short-term studies of this sort (Hoffenberg et al, 1966).

3. Liver Perfusion

The perfused liver provides investigators with an extremely useful tool for examining many facets of its behaviour under controlled conditions, not possible in the intact animal and much more satisfactorily than in tissue cultures. Liver perfusion became established as a laboratory method for research following the work of Miller et al (1951) and Brauer et al (1951).
Details of technique have varied with investigators; however, the principle remains the same. There are two different approaches. The first is the 'in situ' technique, where the liver remains in the carcass, the whole body of the animal being placed in the perfusion cabinet. The second method commonly used involves removal of the liver from the carcass and perfusing it on a dish in the cabinet. The former technique has several advantages: it obviates the necessity of handling the liver, the correct positioning of the liver is ensured and the ischaemic time is reduced by omitting the dissection of the liver from the carcass.

The technique used in this study involves cannulation of the portal vein, cannulation of the inferior vena cava above the inflow of the hepatic veins, cannulation of the bile duct and ligation of the inferior vena cava (just above the inflow of the renal vessels) and hepatic artery (Fig. 9).

The perfusion is carried out in a temperature and humidity controlled cabinet. The liver is perfused with a medium containing adequate nutrients (see appendix) and having a suitable oxygen carrying capacity, maintained at physiological pressure, pH, oxygen and carbon dioxide tension. Microscopy has been used to verify cellular integrity.

This simple experimental model provides the investigator with a tool in which the effects of varying available specific nutrients or hormones, together or separately, can be observed, without the interference of the systemic variations that would be the consequence of these changes in the intact animal. Perfusion was carried out by the author as described by Hems et al (1966). Full details are described in the appendix.
Figure 9. Diagramatic illustration of liver perfusion technique.

LV = left ventricle
HA = hepatic artery

Cannula in right atrium
Heart, L.V.

Partially Hepatectomized Liver

Cannulated Bile duct
H.A. Cannulated Portal vein

Tie
IVC
Kidney
Aorta

Bile
MATERIALS AND METHODS
MATERIALS AND METHODS

The principles of methodology have been discussed. The detailed methodology is given in the appendix. Essential features are summarized.

Male Wistar rats, weighing between 250-300 g. housed in individual cages under controlled conditions of temperature, humidity and lighting were permitted water ad libitum and unless otherwise stated had free access to a balanced diet.

Partial hepatectomies were performed under diethyl-ether anaesthesia with the removal of the median and left lateral lobes as described by Higgins and Anderson (1931) - see appendix. Sham operations consisted of laparotomy and palpation of the liver only.

Radioactive Materials

All the radioactive materials used in these experiments were obtained from the Radio Chemical Centre, Amersham, England.

1. Sodium $\left(^{14}\text{C}\right)$ carbonate - specific radioactivity 58.9 mCi/mM.
2. Sodium $\left(^{131}\text{I}\right)$ iodide - specific radioactivity 40 mCi/mL.
3. Sodium $\left(^{125}\text{I}\right)$ iodide - specific radioactivity 100 mCi/mL.
Measurement of plasma albumin levels (in vivo) following partial hepatectomy.

Eighteen rats were divided into three groups: control, sham-operated and partially-hepatectomized in such a way that each control had a sham-operated and a partially-hepatectomized partner of similar weight.

On day zero, partial hepatectomies were performed on the six rats in that group. They were weighed post-operatively and returned to their individual cages where they received water ad libitum and in which there was a pre-weighed amount of food contained in a vessel designed to minimize spillage. The animals had become accustomed to feeding from these vessels prior to surgery.

On day one the sham operations were performed and that group of rats weighed post-operatively. The control animals were weighed and they and the sham operated animals were given exactly that amount of food which their partially-hepatectomized partners had eaten during the preceding 24 hours. Each day animals were weighed and each trio (partially-hepatectomized, sham operated and control) consumed the same weight of food as indicated above.

In the first experiment the partially-hepatectomized animals were exsanguinated on day 3, and the sham operated and control rats exsanguinated on day 4. In the second experiment the same procedure was adopted, except that the animals were exsanguinated on days 10 and 11 respectively.

Plasma albumin was estimated by the method of Fernandez et al, (1966).
Measurement of albumin synthesis rates (in vitro) following partial hepatectomy (including effects of amino acids and hydrocortisone)

Perfusion.

A perfusion cabinet designed to house isolated liver perfusions at constant temperature (37°C) and humidity was built in the workshop of the Department of Medicine, University of Cape Town. The livers were perfused using the technique described by Hems et al (1966). The details of perfusion technique and perfusate constituents are given in the appendix. Portal pressure was kept constant at 15 cm of water and pH was maintained at 7.35 - 7.45 by addition of aliquots of 4.2% sodium bicarbonate as indicated by a continuously reading pH meter in the reservoir. $pO_2$ and $pCO_2$ were maintained at 150 - 175 and 35 - 40 mm of mercury respectively.

Production of urea and glucose was determined by measuring their levels in the perfusate before and after perfusion on a technicon auto-analyser (Technicon Auto Analyzer, Technical Instrument Co. Ltd., London, England) with appropriate standards. Livers were not considered to be viable in the presence of any of the following: inadequate bile, urea, glucose production or perfusate flow rate; liver swelling or visible infarcts.

The author is aware that albumin synthesis measured in liver perfusions does not reflect realistic (in vivo) rates. However, since the purpose of the project was not to measure absolute albumin synthesis rates but to compare these rates under different conditions, the author felt that the technique was justified. In retrospect the results obtained from the liver perfusions have been validated by those from the in vivo studies.
All perfusions were carried out between 10.00 a.m. and noon to avoid variations due to circadian rhythms and in groups of partially hepatectomized, sham operated and control, each group of 3 consuming the same weight of food during the pre-perfusion period. The perfusions were performed at 6, 12, 18, 22, 72 hours and 7 days after partial hepatectomy or sham operation. Sham operations were not performed in the 72 hour and 7 day groups. At the end of each perfusion the liver was flushed out with cold normal saline excised from the carcass, dried between two pieces of gauze and weighed. The liver was then placed in a dry oven (120°) and weighed repeatedly until no change in weight occurred. The perfusate was spun down and the plasma deep frozen until assayed.

**Synthesis Rate**

Sodium (\(^{14}\)C) carbonate was added to the reservoir after 30 minutes of perfusion by means of a constant infusion pump, 250 uCi being infused over a period of 2 hours. Samples of perfusate were taken at the start and finish of the perfusion.

Albumin synthesis rate was measured by the method of McFarlane (1963a) and Reeve et al (1963). The formula used was equation No. 6 (see Principles of Methodology). The specific radioactivity of urea and of albumin was determined from the pre- and post-samples. Albumin was measured by the method of Fernandez et al (1966).

Urea specific activity was measured on deproteinized plasma samples, incubated with urease to produce \(^{14}\)C\(_2\)O\(_2\) which was then released by the addition of acid.
The volume of gas produced was measured manometrically on a high vacuum gas train, collected in phenylethylanine-methanol and counted in a PPO-POPOP toluene scintillator in a Beckman Automatic Scintillation Spectrometer. Samples were counted to an error of less than 3%. The specific activity of the guanidine carbon of arginine in albumin was obtained by extraction of albumin from the perfusate by the acid ethanol method of Korner and Debro (1956). After acid hydrolysis at 110°, the sample was passed through a column of deacidite FF resin in the bicarbonate form to separate arginine which was then incubated with arginase to produce urea. The sample was then treated as for urea. Urea was measured by a modification of Nessler's method (Henry, 1966).

(a) **Effect of supplementary amino acids on albumin synthesis rate.**

The effect of amino acids on albumin synthesis was examined only in the group 12 hours after partial hepatectomy. In addition to the Sodium (¹⁴C) carbonate, arginine, asparagine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophan and valine were added together to the perfusate, before commencing with the perfusion, to a concentration of 10 times the normal peripheral blood concentration as previously defined (Kirsch et al, 1968b). Details are given in the appendix. Only L-isomers of amino acids (British Drug Houses, Poole, Dorset, U.K.) were added to the perfusate.
(b) Effect of hydrocortisone on albumin synthesis rate after partial hepatectomy.

The effect of hydrocortisone on albumin synthesis was examined only in the group 12 hours after partial hepatectomy. In addition to the Sodium (¹⁴C) carbonate, 30 minutes after the start of the perfusion, 2 mg. hydrocortisone (hydrocortisone sodium succinate injection of B.P. Glaxo Laboratories Ltd., Greenford, England) was added to the reservoir as a bolus and 0.949 mg/hr. for 2 hours as a constant infusion.

3. Measurement of albumin synthesis rates (in vivo) following partial hepatectomy (including effects of supplementary amino acids)

Each experiment was performed in groups of three: partially hepatectomised; partially hepatectomised to whom 10 times the normal peripheral blood concentration of amino acids (see (a) above) was administered orally immediately post-operatively (details are given in the appendix); sham operated. All animals were "triple-fed" (as in 1 and 2 above) during the interval between operation and the start of the experiment and were given drinking water containing 0.008% sodium iodide for at least 24 hours prior to, and throughout, the experiment to block thyroidal uptake of radio-iodine released by breakdown.

Sixteen hours after operation a mixture of ¹⁴C-carbonate (250 µCi) and ¹²⁵I labelled albumin (10uCi) was injected into the tail vein of rats. The simultaneous injection of labelled protein enabled plasma volume measurements to be made.
In addition, by comparing the radio-iodine activity of the 10 minute sample with one taken at the end of the experiment 6 hours after injection, the albumin specific activity at 6 hours could be corrected to $t_0$. Starting 4 hours after the injection of carbonate, the rats were bled at half-hourly intervals, 0.1 ml of whole blood being taken from the tail into a pipette (thus avoiding extensive blood loss) and placed directly into 0.1 ml of 1mgm/ml stable urea and then frozen. At 6 hours the animals were exsanguinated by cardiac puncture, 0.1 ml of this final sample was placed directly into 0.1 ml of 1 mgm/ml stable urea and frozen. Plasma was separated from the remaining blood and stored frozen until assayed. Stable urea and albumin solutions were added to the samples to ensure adequate carbon dioxide volumes, appropriate corrections being made in the calculations. Serum urea estimations on the final sample were done by the Diacetyl Monoxime method as it was observed that the iodine in the water administered before and during the experiment interfered with estimation by Nessler's method which makes use of iodine.

Albumin synthesis was measured by the method of McFarlane (1963a) and Reeve et al, (1963) as described in 2 above. The formula used is equation No. 5 (see Principles of Methodology). The specific radioactivity of urea was calculated by extrapolating the slope of the ($^{14}$C) urea specific radioactivity curve to zero time. The hypothetical specific radioactivity of albumin was obtained by extrapolation to zero time, correcting the 6 hour volume by a factor derived from the distribution and catabolism of the injected ($^{125}$I) albumin. The urea synthesis rate was obtained from the slope of the semi-log plot of ($^{14}$C) urea specific activity versus time.
4. **Measurement of albumin catabolic rates (in vivo) after partial hepatectomy.**

Albumin for this purpose was fractionated from rat plasma by the polyethylene glycol method of Polson and Parker (1973). Iodination with $^{131}\text{I}$ or $^{125}\text{I}$ was achieved by the iodine monochloride method of McFarlane (1958) residual free iodine being removed by passage through an anion-exchange resin column or by dialysis against distilled water. At least 99% labelling efficiency was obtained by mean substitution levels of 1 atom iodine per molecule albumin. Trichloroacetic acid precipitation invariably showed less than 1% free iodine in the final sample. Samples were sterilized by Seitz filtration with the addition of small amounts of carrier albumin or plasma to reduce losses and to protect against radiation damage. Final preparations were checked by cellulose-acetate electrophoresis to ensure absolute chemical purity and homogeneity of the labelled product.

For at least 24 hours prior to the injection and throughout the experiment, rats were given drinking water containing 0.008% sodium iodide to block thyroidal uptake of radio-iodine released by breakdown.

Eighteen rats were divided into three groups and food intake was carefully controlled as described above in 1 and 2.

In the first experiment partial hepatectomies were performed on day 0. Immediately after operation 0.5 ml of iodinated albumin containing 15 - 20 $\mu\text{Ci}$ of $^{131}\text{I}$ was administered intravenously, following which whole body radioactivity was assayed by placing the rats in a well ventilated tin, which was counted in a ring of 6 matched Geiger-Muller tubes to represent 100% of the administered dose.
Syringes were weighed before and after administration for accurate dose determination. Fifteen minutes after administration of the $^{131}$I $0.8$ ml of venous blood was withdrawn from the tail for the estimation of total blood volume and $100\%$ administered dose. On day 1 the sham operations were performed and they and the control group were injected with $^{131}$I and samples taken as above. $0.8$ ml venous blood for estimation of plasma radioactivity and whole body counts were obtained every 24 hours, the partially hepatectomized group being exsanguinated on day 10 and the sham operated and control groups on day 11. A standard solution was made up on day zero consisting of a 1 in 1,000 dilution of the $^{131}$I dose. This was counted daily to enable allowance for natural radioactive decay to be made.

Since intravenously administered albumin does not reach equilibrium within the extravascular space for approximately 48 hours (Sterling, 1951), the above experiment does not provide information regarding the immediate change in albumin catabolic rate following partial hepatectomy. A second series of experiments was designed to try to get this information.

Here the partially hepatectomized group were injected intravenously with $0.5$ ml of iodinated albumin containing $15 - 20$ $\mu$Ci of $^{131}$I on day 0, the sham operated animals and controls being injected intravenously with the same amounts of $^{131}$I on day 1. Immediately after intravenous administration of $^{131}$I, whole body counts were obtained to represent $100\%$ of the administered dose. On day
2 partial hepatectomies were performed on the partially hepatectomized group and immediately post-operatively they were injected intravenously with 0.5 ml of albumin iodinated with $^{125}\text{I}$, 15 - 20 $\mu\text{Ci}$. On day 3 the sham operations were performed and this group injected intravenously, immediately post-operatively, with the same amount of $^{125}\text{I}$ as were the control group. Fifteen minutes after the intravenous administration of $^{125}\text{I}$ 0.8 ml of venous blood was withdrawn for estimation of total blood volume. Separation of the contributions of $^{125}\text{I}$ and $^{131}\text{I}$ was achieved by appropriate voltage discrimination. 0.8 ml of venous blood and whole body counts were obtained every 12 hours, the partially hepatectomized group being exsanguinated on day 5 and the sham operated and control groups on day 6, that is 72 hours after operation. A standard solution was made up as in the first experiment and counted every 12 hours. The serum from all samples was deep frozen until assayed. The plasma albumin was determined from the end sample by the method of Fernandez et al (1966).

Catabolic rate was calculated from the ratio of daily fall in whole body radioactivity and mean plasma specific activity during the corresponding 12 or 24 hours. This ratio defined the fraction of plasma albumin catabolized per day; the product of this fraction and plasma albumin pool provided an absolute catabolic rate (mg albumin per day).
RESULTS
RESULTS

(1) Plasma albumin levels after partial hepatectomy in vivo

These results and those following sham operation together with controls, are shown in Table 1 and Fig. 10. On day 3 the difference between the plasma albumin levels of the controls and the sham operated animals is significant ($p < 0.001$) while there is no significant difference between the sham operated and partially hepatectomized animals. On day 10 the difference between the control and sham operated animals is no longer significant, while that between the control and partially hepatectomized animals remain so ($p < 0.001$). At this time the difference between the partially hepatectomized and sham operated animals has become significant ($0.02 > p > 0.01$).

(2) Albumin synthesis rates following partial hepatectomy in vitro (including effects of amino acids and hydrocortisone.)

These results are shown in Table 2 and Figs. 11 and 12 and the statistical significance of these results is set out in Table 3. It can be seen that the albumin synthesis rate falls significantly after the partial hepatectomy both in terms of dry liver and animal weight, until 22 hours after partial hepatectomy when the albumin synthesis rate has recovered in terms of dry liver weight to such an extent that it is no longer significantly different from the sham operated animal.
a) **Effect of supplementary amino acids on albumin synthesis rate.**

The effect of adding 10 times the normal plasma concentration of eleven amino acids and the effect of adding cortisone to the perfusate is seen in Table 4 and Fig. 13 and the statistical significance of these results is set out in Table 5. The difference between the albumin synthesis rate of the partially hepatectomized liver and that after partial hepatectomy with added amino acids is significant both in terms of dry liver and animal weight. The increase in the albumin synthesis rate of the partially hepatectomized liver by adding amino acids to the perfusate is such that when considered in terms of dry liver weight there is now no significant difference between it and the sham operated animal (Table 5).

b) **Effect of hydrocortisone on albumin synthesis rate after partial hepatectomy.**

The increase in albumin synthesis rate of the partially hepatectomized animal following the addition of cortisone to the perfusate is not significant and the albumin synthesis rate of the sham operated animals remains significantly different from that of the partially hepatectomized group (Table 5).

(3) **Albumin synthesis rate following partial hepatectomy (in vivo) including effects of supplementary amino acids.**

These results are shown in Table 6 and Fig. 14 and the statistical significance set out in Table 7. The results obtained from the in vivo experiments mirror almost exactly
those obtained from the in vitro experiments, the only
difference being that in vivo the partially hepatectomized liver
is able to synthesize albumin at a rate not significantly
different from that of the sham operated animals when considered
in terms of dry liver weight. Further, these results reveal that
in vivo the administration of amino acids is able to stimulate
the partially hepatectomized liver to greater rates of albumin
synthesis such that this rate now becomes significantly higher
than those of the partially hepatectomized animal without
amino acids, both in terms of the whole animal and dry liver
weight; and in the sham operated animal, in terms of dry liver
weight.

In addition, the serum albumin, serum urea, 0-6
hour albumin guanidine carbon specific radioactivity ratio
and the fractional synthesis rate of urea were measured to
ensure that observed changes in albumin synthesis rates were
not in any way artificial (Table 8). The similarity of
these results in all three experimental groups confirms the
validity of the conclusions drawn from the results.

(4) **Albumin catabolic rates following partial
hepatectomy in vivo.**

These results are given in Table 9 and illustrated
in Fig. 15 and the statistical significance is seen in
Table 10. The albumin catabolic rate of the partially
hepatectomized animals is significantly lower than the
catabolic rates of the sham operated and control animals,
in both sets of experiments.
<table>
<thead>
<tr>
<th>Interval after partial hepatectomy</th>
<th>3 Days</th>
<th>10 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of experiment *</td>
<td>Control</td>
<td>P.H. ++</td>
</tr>
<tr>
<td>Mean plasma albumin level (g%)</td>
<td>2.99 ± 0.09</td>
<td>2.29 ± 0.03</td>
</tr>
</tbody>
</table>

* Six animals in each of the three groups

+ for statistical analysis of results see text

++ partial hepatectomy
Fig 10. Plasma Albumin Levels Following Partial Hepatectomy

(Controls, Shams and Partially Hepatectomised Animals "Triple" Fed)
**TABLE 2.**

ALBUMIN SYNTHESIS RATES (mg per hour) - IN VITRO (PERFUSION STUDY)

<table>
<thead>
<tr>
<th>Interval after Partial Hepatectomy</th>
<th>Control 6 hours</th>
<th>Sham 12 hours</th>
<th>Control 22 hours</th>
<th>Sham 72 hours</th>
<th>Sham P.H. 7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of experiment</td>
<td>P.H.†</td>
<td></td>
<td>P.H.†</td>
<td></td>
<td>P.H.†</td>
</tr>
<tr>
<td>No. of experiments</td>
<td>9</td>
<td>6</td>
<td>7</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Results expressed per 300 g rat</td>
<td>2.66**</td>
<td>0.36</td>
<td>2.47</td>
<td>0.25</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>± 0.48+++</td>
<td>± 0.13</td>
<td>± 0.25</td>
<td>± 0.04</td>
<td>± 0.20</td>
</tr>
<tr>
<td>Results expressed per g dry liver</td>
<td>1.09++</td>
<td>0.36</td>
<td>0.97</td>
<td>0.26</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>± 0.17+++</td>
<td>± 0.13</td>
<td>± 0.11</td>
<td>± 0.04</td>
<td>± 0.10</td>
</tr>
</tbody>
</table>

† P.H. - Partial Hepatectomy
++ Mean albumin synthesis rate
+++ Standard error of mean
Fig 11 ALBUMIN SYNTHESIS RATES (IN VITRO)
in mg/hr/300g rat

Control 2.66
Sham 2.47

6 hrs 12 hrs 22 hrs 72 hrs 7 days
P.H. 0.36 P.H. 0.25 P.H. 0.71 P.H. 1.02 P.H. 1.18

TIME AFTER PARTIAL HEPATECTOMY
*P.H. = Partial Hepatectomy
Fig. 12 ALBUMIN SYNTHESIS RATES (IN VITRO)
mg/hr/g dry liver

<table>
<thead>
<tr>
<th>Time</th>
<th>Control 1.09</th>
<th>Sham 0.97</th>
<th>Sham 0.85</th>
<th>Sham 0.76</th>
<th>P.H. 0.59</th>
<th>P.H. 0.58</th>
<th>P.H. 0.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 hrs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>12 hrs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22 hrs</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

TIME AFTER PARTIAL HEPATECTOMY
*P.H. = Partial Hepatectomy
<table>
<thead>
<tr>
<th>Interval after Partial Hepatectomy</th>
<th>6 hours</th>
<th>12 hours</th>
<th>22 hours</th>
<th>72 hours</th>
<th>7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per 300g Rat</td>
<td>Per 300g dry liver</td>
<td>Per 300g Rat</td>
<td>Per 300g dry liver</td>
<td>Per 300g Rat</td>
</tr>
<tr>
<td>Expression of results</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control vs Partial Hepatectomy</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>0.005</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>&gt;p&gt;</td>
<td>&gt;p&gt;</td>
<td>&gt;p&gt;</td>
<td>&gt;p&gt;</td>
<td>&gt;p&gt;</td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td>0.01</td>
<td>0.01</td>
<td>0.05</td>
<td>0.005</td>
</tr>
<tr>
<td>Sham vs Partial Hepatectomy</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>N.S.++</td>
</tr>
<tr>
<td></td>
<td>&gt;p&gt;</td>
<td>&gt;p&gt;</td>
<td>&gt;p&gt;</td>
<td>&gt;p&gt;</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>-</td>
</tr>
<tr>
<td>Control vs Sham</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

+ Student's 't' test
++ N.S. - Not significant
### TABLE 4.

**EFFECT OF AMINO ACIDS AND CORTISONE ON ALBUMIN SYNTHESIS RATE** *(mg per hour) (IN VITRO)*

<table>
<thead>
<tr>
<th>No. of experiments</th>
<th>No addition to perfusate</th>
<th>10 x 11 amino acids added to perfusate</th>
<th>Cortisone added to perfusate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Results expressed per 300 g rat</td>
<td>0.25 ± 0.04**</td>
<td>0.517 ± 0.12</td>
<td>0.31 ± 0.03</td>
</tr>
<tr>
<td>Results expressed per g dry liver</td>
<td>0.26 ± 0.04**</td>
<td>0.517 ± 0.09</td>
<td>0.30 ± 0.03</td>
</tr>
</tbody>
</table>

* Experiments performed on animals 12 hours after partial hepatectomy

** Mean albumin synthesis rate  
* Standard error of mean
Fig. 13 ALBUMIN SYNTHESIS RATES (IN VITRO)
mg/hr/g dry liver

Effect of adding
1. hydrocortisone to perfusate†
2. amino acids

Control 1.09
Sham 0.85

P.H. + 10×11 amino acids 0.52

*P.H. Partial Hepatectomy
†12 hrs after P.H.
### TABLE 5.

**STATISTICAL SIGNIFICANCE**: EFFECT OF AMINO ACIDS AND CORTISONE ON ALBUMIN SYNTHESIS RATE IN VITRO

<table>
<thead>
<tr>
<th>Expression of results</th>
<th>P.H. $^*$ v P.H. with 10 x 11 amino acids</th>
<th>P.H. with 10 x 11 amino acids v sham</th>
<th>P.H. v P.H. with cortisone</th>
<th>P.H. with cortisone v sham</th>
</tr>
</thead>
<tbody>
<tr>
<td>per 300 g rat</td>
<td>p&lt;0.05</td>
<td>p&lt;0.001</td>
<td>N.S. (p&gt;0.3)</td>
<td>p&lt;0.0005</td>
</tr>
<tr>
<td>per g dry liver</td>
<td>p&lt;0.0125</td>
<td>N.S. $^*$ (p&gt;0.3)</td>
<td>N.S. (p&gt;0.7)</td>
<td>p&lt;0.0005</td>
</tr>
</tbody>
</table>

$^*$ Student's 't' test

$^{++}$ Experiments performed on animals 12 hours after partial hepatectomy - cf table 2 for sham operated values.

$^* N.S.$ - not significant

$^* $ Partial hepatectomy
<table>
<thead>
<tr>
<th></th>
<th>P.H.†</th>
<th>P.H. to whom 10 x 11 amino acids administered</th>
<th>Sham Operated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of experiments</td>
<td>5</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Results expressed per 300 g rat</td>
<td>4.89**, ± 0.35***</td>
<td>7.03, ± 0.31</td>
<td>10.18, ± 0.76</td>
</tr>
<tr>
<td></td>
<td>3.07, ± 0.34</td>
<td>4.31, ± 0.23</td>
<td>3.43, ± 0.33</td>
</tr>
</tbody>
</table>

+ P.H. - Partial hepatectomy
++ Mean albumin synthesis rate
+++ Standard error of mean
* Experiments performed on animals 16 hours after partial hepatectomy.
Fig. 14
ALBUMIN SYNTHESIS RATES (IN VIVO)
The effect of adding amino acids†

mg/hr/300g rat
P.H. + amino acids - 7.03
Sham - 10.18

mg/hr/g dry liver
P.H. - 3.07
P.H. + amino acids - 4.31
Sham - 3.43

* P.H. = Partial Hepatectomy
† 16 hrs after P.H.
TABLE 7.

<table>
<thead>
<tr>
<th>Statistical Significance* : Albumin Synthesis Rates : (Including Effects of Amino Acids) in Vivo</th>
<th>per 300g rat</th>
<th>per g dry liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partial hepatectomy vs Partial hepatectomy + 10 x 11 amino acids</td>
<td>p&lt;0.0025</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Partial hepatectomy vs Sham operated</td>
<td>p&lt;0.0025</td>
<td>N.S. ++ (p&gt;0.2)</td>
</tr>
<tr>
<td>Partial hepatectomy + 10 x 11 amino acids vs Sham operated</td>
<td>p&lt;0.0025</td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>

* Student's 't' test
++ N.S. - Not significant
* Experiments performed on animals 16 hours after partial hepatectomy.
<table>
<thead>
<tr>
<th></th>
<th>Partial Hepatectomy</th>
<th>Partial Hepatectomy</th>
<th>Sham operated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+ 10 x 11 amino acids</td>
<td></td>
</tr>
<tr>
<td>Serum albumin (g%)</td>
<td>2.28</td>
<td>2.25</td>
<td>2.17</td>
</tr>
<tr>
<td></td>
<td>± 0.05*</td>
<td>± 0.03</td>
<td>± 0.04</td>
</tr>
<tr>
<td>Serum urea (mg%)</td>
<td>44.48</td>
<td>49.53</td>
<td>39.00</td>
</tr>
<tr>
<td></td>
<td>± 3.56</td>
<td>± 6.46</td>
<td>± 4.31</td>
</tr>
<tr>
<td>0 - 6 hour albumin</td>
<td>48.24</td>
<td>48.29</td>
<td>49.26</td>
</tr>
<tr>
<td>guanidine carbon specific radio activity ratio</td>
<td>± 2.61</td>
<td>± 1.04</td>
<td>± 1.65</td>
</tr>
<tr>
<td>Fractional synthesis rate of urea (%/hr)</td>
<td>29.06</td>
<td>29.56</td>
<td>29.53</td>
</tr>
<tr>
<td></td>
<td>± 2.14</td>
<td>± 2.60</td>
<td>± 1.98</td>
</tr>
</tbody>
</table>

* Standard error of mean
### ALBUMIN CATABOLIC RATES (mg/hour/300g rat)

<table>
<thead>
<tr>
<th>Group</th>
<th>Measurements taken every 12 hours for 72 hours</th>
<th>Measurements taken every 24 hours for 10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11.22 ± 0.97</td>
<td>9.47 ± 0.38</td>
</tr>
<tr>
<td>Sham operated</td>
<td>12.06 ± 1.31</td>
<td>11.04 ± 1.14</td>
</tr>
<tr>
<td>Partial hepatectomy</td>
<td>7.36 ± 0.76</td>
<td>7.47 ± 0.42</td>
</tr>
</tbody>
</table>

Six animals in each of the three groups

+ Mean catabolic rate ± Standard error of mean
Fig. 15
ALBUMIN CATABOLIC RATES (IN VIVO)
in mg/24 hr/300g rat

<table>
<thead>
<tr>
<th>Group</th>
<th>Sham</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>12.06</td>
<td>11.22</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>11.04</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P.H.*
7.36
7.48

Calculated from samples taken every 12 hrs for 72 hrs
Calculated from samples taken every 24 hrs for 10 days

*P.H. = Partial Hepatectomy
<table>
<thead>
<tr>
<th></th>
<th>12 hourly samples in 72 hours</th>
<th>24 hourly samples in 10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control vs Partial Hepatectomy</td>
<td>0.02&gt;p&gt;0.01</td>
<td>0.005&gt;p&gt;0.001</td>
</tr>
<tr>
<td>Control vs Sham</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>Sham vs Partial Hepatectomy</td>
<td>0.02&gt;p&gt;0.01</td>
<td>0.02&gt;p&gt;0.01</td>
</tr>
</tbody>
</table>

+ Student's 't' test
N.S. - Not significant
DISCUSSION
DISCUSSION

Experimental work concerning albumin synthesis in animals following partial hepatectomy has produced conflicting results when these results are expressed as synthesis in terms of remaining liver weight. Some workers have reported a fall in the albumin synthesis rate (Mutschler and Gordon 1966; Murray-Lyon and Muller-Eberhard 1973) others an increase (Majumdar et al 1967) while a third group report no change in the albumin synthesis rate (Guidotti et al 1959; Braun et al 1962; Rosenoer et al 1970; Schreiber et al 1971).

Despite these differences in synthesis rates per unit remnant liver weight, all studies agree that after partial hepatectomy there is a fall in plasma albumin concentration. This has been confirmed in man (Islami et al 1956; Pack and Molander, 1960; McDermott et al, 1963; Monaco et al 1964; McDermott and Ottinger 1966; Almersjö and Benmark 1969; Aronsen et al, 1969; Dillard, 1969; Flatmark et al 1973).

The author's results are in agreement with this observation. Following partial hepatectomy the plasma albumin levels decreased from 2.77 to 2.09 (p < 0.001). Food intake was controlled in all groups to exclude changes due to diet. The fall of plasma albumin seen in the partially hepatectomized animals after three days (2.99 - 2.29 g%) was mirrored to almost the same extent by the sham operated animals (2.99 - 2.42 g%). By the tenth day, however, the plasma albumin of the partially hepatectomized animals had fallen further to 2.09 g%, while that of the sham operated group had been almost completely restored (2.58 g%) to the control levels (2.77 g%).
Since at no time after partial hepatectomy is there any significant difference of the albumin synthesis and catabolic rates between the control and sham-operated animals (see Tables 3 and 9 and Figs. 12 and 15), some other mechanism must be instrumental in the fall of plasma albumin. Kirsch et al (1968b) demonstrated an immediate fall in the albumin synthesis rate following protein deprivation. Catabolic rates remained within the normal range until the plasma albumin pool diminished after which catabolism of albumin rapidly decreased. The author has attributed the fall in the plasma albumin of the control animals (which were "triple-fed") during the 10 day post operative period (2.99 - 2.77 g%) to protein-calorie deprivation for during the entire period their partially hepatectomized partners ate considerably less than animals fed ad libitum.

The initial fall in the plasma albumin of the sham operated animals is attributed to the sequestration of albumin into non-exchangeable extravascular compartments that has been shown to follow surgery (Mourisden and Faber, 1966; Mourisden, 1967; Hoye et al, 1972). Mourisden and Faber (1966) and Mourisden (1967) have shown an increased elimination of $^{131}$I labelled albumin from plasma of patients undergoing major surgery and have demonstrated a "migration of albumin into the oedema of the operative field". Hoye et al (1972) investigating fluid volume and albumin synthesis rates in patients undergoing major and minor surgery, found a reduction in plasma volume during major surgery disproportionate to the reduction in total red blood cell volume.
This was associated with a decrease in both the lymph flow in the thoracic duct and the albumin concentration of the lymph together with a fall in plasma albumin and the total exchangeable albumin pool.

There is a highly significant increase in protein concentration and excretion in the urine in patients following surgery (MacBeth and Pope, 1968), this anomaly being greater in those patients undergoing major surgery.

Besides these observations, other less well defined factors are involved in circulating albumin loss and include: Loss into the abdominal cavity (Jarnum, 1961), increased capillarity permeability (Birke et al, 1959/60), intravenous infusion (Wasserman and Mayerson, 1952; Kaijser and Reiger, 1968) and an increase in fractional catabolic rate (Grossman et al, 1960).

The fall in the plasma albumin in the sham operated animals is therefore attributed initially to the sequestration of albumin into non-exchangeable extravascular compartments together with the other factors discussed and this is perpetuated by protein calorie deprivation as happens in the control group.

The evidence that plasma albumin falls following surgery not related to the liver, further complicates the matter of investigating albumin metabolism following partial hepatectomy. By using sham operated animals the author has demonstrated the degree to which sequestration of albumin and the other mechanisms discussed above which are instrumental in the fall of plasma albumin following major surgery, contribute to the fall in plasma albumin following partial hepatectomy.
Following partial hepatectomy the albumin synthesis rate falls significantly. In vitro this fall is greatest at 12 hours and least at 22 hours after the operation, when the recovery of the albumin synthesis rate of the liver is such that when the results are expressed in terms of dry liver weight, the synthesis rates of the partially hepatectomized and sham operated animals are no longer significantly different. In vivo the albumin synthesis rates of the sham operated and partially hepatectomized livers (in terms of dry liver weight) are not significantly different 16 hours after operation. However, it must be emphasized that only one third of the original hepatic mass remains so that despite the return of the albumin synthesis rate to control levels in terms of unit hepatic mass, the total albumin synthesis is much less than it would be if the intact liver was synthesizing albumin; this accounts for the fall in plasma albumin. One explanation of the apparent earlier return of the albumin synthesis rate of the partially hepatectomized liver to that of the sham operated animal IN VIVO may be that in the intact animals amino acids and hormones are mobilized from the extrahepatic stores in order to maintain albumin synthesis per unit hepatocyte. In addition, it is not expected that in the artificial situation of liver perfusion the organ will perform as efficiently as it does in vivo.

Other workers (Rosenoer et al, 1970) using isolated rat liver perfusions, found that 72 hours after partial hepatectomy, the albumin synthesis rates were significantly
increased when expressed in terms of both animal weight and dry liver weight. In contrast, in the in vitro studies reported here the albumin synthesis rate was still significantly depressed when compared to control animals both in terms of animal weight and dry liver weight 72 hours after partial hepatectomy. At no time during this period was an elevation of albumin synthesis rate found in the partially hepatectomized animals. These results are more in keeping with those obtained from the investigations of serum albumin levels and albumin catabolic rates in this experimental situation.

It is important to stress that all groups of animals were "triple-fed" during the experiments carried out to determine albumin synthesis rates, as well as in those investigating serum albumin levels and albumin catabolic rates after partial hepatectomy.

Rothschild et al (1966) have postulated an extravascular osmotic regulatory system controlling albumin synthesis and it is known that albumin is sequestered in extravascular compartments post-operatively. The raised extravascular osmotic pressure caused by this sequestration of albumin may be one mechanism whereby the albumin synthesis rate is depressed in the early stages after operation. Although the albumin synthesis rate of the partially hepatectomized and sham operated animals are no longer significantly different 18-22 hours post operatively (when expressed in terms of dry liver weight), this hypothesis is not supported by the highly significant differences between these two groups at the earlier intervals.
post-operatively, even when considered in terms of liver weight.

Because of the integral role of amino acids in liver protein synthesis, the investigation of their effect on albumin synthesis following partial hepatectomy, was undertaken. Christensen et al (1948) and Braun et al (1962) showed that the levels of amino acids in the liver are increased at an early stage after partial hepatectomy and it has been demonstrated that amino acids are mobilized for plasma protein synthesis in in vivo experiments on nephrotic rats. (Drabkin et al, 1962).

These findings were confirmed more recently by Ferris and Clark (1972) who showed a rise in both hepatic and plasma free amino acid concentrations during the first four hours after partial hepatectomy as compared to sham operated animals. Using in vitro techniques Marsh and Drabkin (1958) and Peters (1959) were unable to demonstrate an accelerating effect of added amino acids on the albumin synthesis rates. Clemens (1972) however, has shown an increase in protein synthetic activity in cell free systems after exposure to high concentrations of amino acids, while Kelman et al (1972b) using the isolated perfused rat liver demonstrated the ability of certain amino acids, in high concentration, to restore the albumin synthesis rates of isolated perfused livers from starved rats to normal levels.

The author has clearly demonstrated that by adding 10 times the normal plasma concentration of eleven amino acids the increase in the albumin synthesis rate is highly significant, both in vivo and in vitro.
The amino acids were added, in vitro, to the 12 hour post-partial hepatectomy group since it is at this time that the albumin synthesis rate is most depressed and any effect the amino acids might have would be more readily appreciable. It is possible that if amino acids are added to the group 22 hours post-partial hepatectomy, the albumin synthesis rates may be returned to normal (control) values.

These results indicate that amino acid supplementation enhances not only the absolute amount of albumin synthesis but (in vivo) enables the hepatic remnant to synthesize albumin at rates greater than the sham operated animal in terms of dry liver weight.

Braun et al (1962) have claimed that in the early stages of liver regeneration precedence is given to the biosynthesis of cellular proteins and that after the regenerative process is well advanced, the increased construction of plasma albumin is metabolically favoured. One hypothetical mechanism whereby amino acids stimulate albumin synthesis could be that as a result of the redistribution of cellular amino acids following partial hepatectomy, the supply of primary substrate for albumin synthesis is limiting (Fig. 16).

Alternatively, if depression of albumin synthesis was due to a limited availability of specific tRNA's or amino acid tRNA synthetases, this restriction could be partly reversed by increasing cellular amino acid concentrations above normal levels (Fig. 17).
Figure 16. Mechanism whereby addition of amino acids may increase albumin synthesis – primary substrate level.
Free amino-acid pool (primary substrate) + Amino-acyl tRNA + Specific synthetases + Specific tRNA → Amino-acyl-tRNA complex

Messenger RNA

Ribosome

Nascent polypeptide

AMINO ACID ACTIVATION

RIBOSOMAL PROTEIN SYNTHESIS

Figure 17. Mechanism whereby addition of amino acids may increase albumin synthesis-ribosomal level.
The observations that amino acids stimulate albumin synthesis could thus be reconciled with either explanation, although the present data are insufficient to indicate which mechanism is in fact the predominant one. Either hypothesis provides an explanation for both the initial fall in the albumin synthesis rate following partial hepatectomy and the ability of amino acids to increase it.

Nolan and Hoagland (1971) have shown (in vitro) that following partial hepatectomy the initial rate of amino acid incorporation into protein in regenerating microsomes is approximately twice that of normals. In the isolated perfused rat liver high concentrations of amino acids are known to maintain aggregation of ribosomes, said to be essential for normal albumin synthesis (Kelman et al, 1972b). However, in livers exposed to ethanol, albumin synthesis is enhanced by addition of amino acids while the ribosomes remain in a disaggregated state (Rothschild et al 1971). The role of ribosomal aggregation therefore remains unclear.

While the mechanism remains obscure, it is of clinical importance that the administration of amino-acids to patients with low serum albumin levels may increase the albumin synthesis rates and so assist in restoring the serum albumin to near normal levels. Recent work by Skillman et al (1976), demonstrating that intravenous infusion of amino acids in patients who had undergone major gastro-intestinal-tract surgery significantly increased albumin synthesis rates when compared to controls who received 5 per cent glucose, suggests that amino acids have a similar effect in man.
Following on the work by this author, Kirsch et al (1976) have demonstrated that keto-analogues of branch-chained amino-acids (leucine, isoleucine and valine) are able to reverse the reduced synthesis rate observed when isolated livers, from well nourished animals were perfused with blood from rats deprived of dietary protein for 48 hours. Furthermore, these keto-analogues increased albumin synthesis per unit dry liver weight to above normal levels when administered orally to rats 16 hours after partial hepatectomy. This is the first demonstration that synthesis of an individual protein may be affected by a non-nitrogenous nutrient. It remains speculative as to whether the keto-analogues act directly or exert their effect only after transamination to amino acids.

Keto-analogues of essential amino acids have been proposed as a method of treating patients with uraemia, portosystemic encephalopathy as well as individuals with carbamyl phosphate synthetase deficiency, (Walser et al, 1973; Maddrey, et al, 1974 and Batshaw et al, 1975). The basic treatment of these patients consists of withholding nitrogenous compounds which results in reduced albumin synthesis (Kirsch et al, 1968a). The finding that keto-analogues are able to increase albumin synthesis rates after partial hepatectomy in the same way as amino acids are able to, may have clinical implications in that by using keto-analogues in patients in whom dietary protein restriction is essential, protein synthesis can still be maintained.
Great interest has been shown in the role of insulin and glucagon in the control of liver cell proliferation. This has provoked much experimental work in this direction and the effect of these hormones on protein synthesis has been studied extensively (Tavill et al, 1975; Miller, 1976).

Physiologically insulin is anabolic; it increases protein synthesis partially by increasing the incorporation of amino acids into proteins (an action that is independent of its effect on glucose metabolism) and partially by the protein sparing action of ensuring adequate intracellular glucose supplies. Insulin is thus a hormone of energy storage: glucagon, on the other hand, is a hormone of energy release. In particular, in this context, the latter stimulates gluconeogenesis from available amino acids.

Using isolated rat liver perfusions Haft and Miller (1958) and Miller (1961) have confirmed that insulin increases albumin synthesis and Miller (1960) confirmed that glucagon was the hormone of protein catabolism in the liver. Tavill et al (1975) using the isolated rat liver perfusion technique showed an "anti-anabolic" role for glucagon in albumin synthesis which could not be prevented by increased amino acid supply or insulin. They further showed that, in contrast, the catabolic effects of glucagon were probably confined to the intracellular proteins and available amino acids and these effects could be inhibited by a relatively low insulin: glucagon molar ratio.
Miller (1976) claims, however, that the unfavourable effects of glucagon on albumin synthesis were reversed by giving insulin simultaneously. It is thus clear that a study of the effect of these hormones on albumin metabolism in the regenerating liver would be a worthwhile line of investigation.

Another factor of importance in relation to albumin synthesis is total hormonal balance. While changes in the availability of specific amino acids released from altered tissue degradation may also play a role in tissue culture, cortisone has been shown to increase albumin synthesis per se (Bancroft et al, 1969). The author was unable to demonstrate any alteration in the albumin synthesis rate following the addition of cortisone to the perfusate. This is in keeping with the findings of Kelman et al (1973) who were able to influence ribosomal profiles by adding 10 times the normal concentration of amino acid to the system, but not by adding the following hormones: hydrocortisone, growth hormone, insulin, tri-iodothyronine and glucagon.

Two sets of experiments were carried out to investigate albumin catabolism following partial hepatectomy - a short-term experiment to investigate the immediate effect of partial hepatectomy on albumin catabolism and a longer term study to allow these changes to be observed for 10 days. There is excellent correlation between the two experiments, both indicating an immediate significant fall in the albumin catabolic rate following partial hepatectomy as the system endeavours to maintain the plasma albumin pool. This finding is in keeping with the findings of Kirsch et al
(1968a) who observed that a fall in the albumin catabolic rate of malnourished rats occurred following the fall in their plasma albumin pools as a result of protein calorie deprivation.

It is thus clear that whatever mechanisms are instrumental in the fall of plasma albumin following major surgery, in the specific case of partial hepatectomy in the rat the most significant factor is a reduction in the albumin synthesis rate, which can be totally corrected by the administration of amino acids or their keto-analogues. Despite the early recovery of the hepatic remnant to synthesize albumin the plasma albumin remains low for at least 10 days because of the reduction in total hepatic mass and hence the total albumin synthesizing machinery.
SUMMARY

This thesis concerns albumin metabolism following partial hepatectomy in the rat. The parameters of albumin metabolism that were studied were:

1. Plasma albumin levels following partial hepatectomy.
2. The effect of partial hepatectomy on the albumin synthesis rate.
3. The effect of supplementary amino acids on the albumin synthesis rate following partial hepatectomy.
4. The effect of hydrocortisone on the albumin synthesis rate following partial hepatectomy.
5. The effect of partial hepatectomy on the albumin catabolic rate.

The plasma albumin levels were studied in vivo while the effect of partial hepatectomy on the albumin synthesis rate, effect of supplementary amino acids and of hydrocortisone on the albumin synthesis rate following partial hepatectomy were all studied both in vivo and in vitro. The effect of partial hepatectomy on the albumin catabolic rate was studied in vivo.

A review of liver cell proliferation has been given and the introduction deals with the inevitable association between studies of albumin metabolism in the partially hepatectomized liver and liver cell proliferation. The clinical relevance of information concerning both liver cell proliferation and albumin metabolism following partial hepatectomy is stressed and the available literature concerning albumin metabolism after partial hepatectomy is reviewed.
In the principles of methodology a detailed account of the method used to measure albumin synthesis is given and it is emphasized that the use of radioactive isotopes in this area is vastly superior to other methods previously used especially the derivation of albumin synthesis rates from catabolic studies of subjects assumed to be in nutritional equilibrium, when synthesis was presumed to equal catabolism. A brief account is given of the work leading up to the method used by the author, described by McFarlane (1963a) and by Reeve et al (1963). Likewise a background is given to the development of the method used in estimating albumin catabolism which was measured using iodine labelled albumin.

The results obtained by the author demonstrate a significant fall in plasma albumin after partial hepatectomy which is not transient as is the fall in the sham-operated animal. The fall in the plasma albumin seen in the "triple-fed" control animals during the 10 day post-operative period is attributed to protein calorie deprivation while the fall in the plasma albumin of the sham-operated animals is attributed to the sequestration of albumin into non exchangeable extravascular compartments together with protein calorie deprivation.

There is a significant decrease in the albumin synthesis rate both in vitro and in vivo in terms of whole animal weight while in terms of dry liver weight there is an initial fall in the albumin synthesis rate in vitro which returns to the rate of the sham-operated animals after 22 hours; in vivo no significant fall occurs in terms of dry liver weight. It is stressed that despite the return of the albumin
synthesis rate to control levels in terms of hepatic mass, the total albumin synthesis is much less than it would be if the intact liver was synthesising albumin, thus accounting for the fall in plasma albumin levels.

The addition of 10 times the normal plasma concentration of 11 amino acids is shown to almost double these values (in vitro), significantly increasing the albumin synthesis rate so that when considered in terms of liver weight there is no significant difference between the partially hepatectomized and the sham-operated animals. In vivo the addition of these amino acids enhances albumin synthesis to such an extent that the partially hepatectomized animal now synthesizes albumin at a rate significantly greater than that of the sham-operated animal.

Hydrocortisone is demonstrated to have no effect on albumin synthesis rates either in vivo or in vitro.

Albumin catabolic rates following partial hepatectomy are shown to fall following the decrease in albumin synthesis rates as the system endeavours to maintain the plasma albumin pool.

Hypothetical mechanisms whereby amino acids may stimulate albumin synthesis following partial hepatectomy are aluded to in the discussion where the clinical importance of the fact that the administration of amino acids to patients with low serum albumin levels may increase the albumin synthesis rate is stressed. It was this discovery by the author which stimulated the research leading to the documentation of the important fact that ketoanalogues of
branch-chained amino acids increase albumin synthesis rates (per unit dry liver weight) to above normal levels when administered orally to rats after partial hepatectomy. The discovery that these non-nitrogenous ketoanalogues, too, can increase albumin synthesis in this situation confirms the hypothesis that these compounds may be useful in the therapy of patients who, for example, are uraemic, have porto-systemic encephalopathy or suffer from carbamyl phosphatase deficiency.

The role of insulin and glucagon in the control of liver cell proliferation is discussed and the importance of further investigation in this direction is mentioned. Possible mechanisms whereby insulin and glucagon may have some control of liver cell proliferation is discussed.

The work described in this thesis, therefore, has expanded the existing literature concerning albumin synthesis following partial hepatectomy in this way:

1. Clarifying the effect of partial hepatectomy on albumin synthesis rates (there being considerable difference of opinion in the literature hitherto).

2. Demonstrating for the first time the beneficial effect of certain amino acids on the albumin synthesis rates in this situation.

3. Elucidating the role of albumin catabolism in the fall in serum albumin which occurs following partial hepatectomy.
APPENDIX
A. LIVER PERFUSION

(1) **Apparatus:**
Set up apparatus as in Fig. 18. Care is taken to ensure that the pressure in the portal vein is exactly 18 cm. perfusate.

(2) **Perfusate:**
Blood was obtained from rats, kept under the same conditions as those perfused, by cardiac puncture under diethyl ether anaesthesia.

1. 56 ml whole blood.
2. 4 ml heparin (1,000 units/ml - heparin injection B.P., Evans Medical Ltd., Speke, Liverpool, England).
3. 25 ml Plasmalyte B (Baxter-Saphar Laboratories Ltd., Johannesburg, South Africa) containing g/100 ml:
   - Sodium Chloride 0.6, Potassium Chloride 0.03,
   - Magnesium Chloride 0.03 and Sodium Bicarbonate 0.23.
4. 2 ml of 4.2% Sodium Bicarbonate.
   The final haematocrit was 25%.

(3) **Operative technique:**
The rat is anaesthetized in a plastic bag into which is passed a mixture of 95% O₂, 5% CO₂, containing 15 - 20% of diethyl-ether. When unconscious the animal is placed on the operating table under continuous anaesthesia.
The abdomen is opened through a mid-line incision and mid-transverse incisions to left and right of the mid-line are made, avoiding the larger vessels. Bleeding is minimized by clamping the major vessels of the abdominal wall with four artery forceps.
a. Tap
b. Clamp
c. Canula in portal vein
e. Constant reading pH meter
f. Filter
h. Bile

o. Oxygenator
p. Pump
r. Reservoir with magnetic stirrer
t. Thermostatically controlled heater
The intestines are then placed to the animal's left, between layers of tissue wetted with warm saline, so that the liver, portal vein, right kidney, inferior vena cava and the bile duct become exposed. The thin strands of connective tissue between the right lobe of the liver and the vena cava are cut and a loose ligature of silk (size 3/0) is placed around the cava above the right renal vein.

Next the bile duct is cannulated by a 30 cm. length of Portex tubing size PP10 cut at an angle to provide a sharp point. The cannula is inserted and pushed to the point where the duct arises from its branches and secured with a silk ligature.

Three loose ligatures are passed around the portal vein at intervals of 3-4 mm. below the point where the vein divides to enter the separate lobes of the liver and a fourth ligature is placed around the vein at a point distal to the liver. This ligature is tied, shutting off the blood supply from the viscera to the portal vein, the portal vein is then incized with a pair of sharp ophthalmic scissors and cannulated with a naso-gastric tube (French size 8, Pharmaseal Incorp. Tao Alta, Puerto Rico) whose tip has been drawn out over a gas-flame and fashioned to be easily introduced into the portal vein. This naso-gastric tube is attached to the end of a conventional intravenous giving set which is delivering Plasmalyte B (see perfusate) warmed to 37°C. The drip is adjusted such that the fluid is continuously running slowly out of the cannula while it is introduced into the portal vein thus avoiding air emboli. Care is taken that at no time is the pressure of the
fluid greater than 18 cms. water. The three loose ligatures are tied securing the cannula in place.

The thorax is opened by a transverse incision just above and along the line of the diaphragm and by two longitudinal incisions towards the head from the two ends of the transverse incision. The chest wall is flapped back towards the head and a large (15 cm) pair of artery forceps is placed along the base of the flap and locked in position. The flap is then cut off. The vagus and phrenic nerves and oesophagus are cut about 1 cm. above the diaphragm to paralyse the diaphragm and to eliminate possible vasoconstrictor effects of the vagus, and a loose ligature is placed around the inferior vena cava close to the heart.

The cannula that is placed in the inferior vena cava is the remainder of the naso-gastric tube described above, whose tip is suitably fashioned. The right atrium is incised sufficiently to allow the cannula to be introduced. It is then passed down the inferior vena cava towards the diaphragm as far as it will go and then tied in position. At this stage, the loose ligature around the abdominal vena cava is tied.

The preparation is then transferred to the perfusion apparatus, care being taken not to allow any air into the cannula in the portal vein. The effluent from the cannula in the inferior vena cava is collected and discarded until the plasmalyte B has been cleared and perfusate is being delivered. The cannula is then placed into the reservoir. The whole operation takes about 10 mins. and the ischaemic time (the period between tying the distal ligature around the portal vein and commencing the flow of perfusate through the liver) is 70-90 seconds.
An indication of the success of the operation is a uniform red colour of the perfused liver, indistinguishable from the colour in vivo. An extended interruption of the liver circulation may cause an initial patchy perfusion, indicated by uneven colouring from which the liver may not completely recover.

At 15 min. intervals 0.3 ml perfusate are withdrawn from the reservoir, through the side arm, and the pH, $pO_2$ and $pCO_2$ measured on an Astrup radiometer. Recordings of bile blow rate, perfusate flow rate, liver appearance are made at the same intervals while ensuring that the cabinet temperature and humidity are maintained. A telethermometer (Beckman) placed between the liver lobes ensures that the liver temperature is constant at 37 °C and a continuously reading pH electrode in the reservoir gives early warning of changes in the perfusate pH.
B. PARTIAL HEPATECTOMY

The operation is performed under diethyl-ether anaesthesia. Through a midline incision reaching 3 or 4 cm posteriorly from the xiphisternum the large median lobe of the liver with the left lateral lobe, is delivered, secured with stout thread and excised. In this way, portions of hepatic tissue ranging from 68-72% of the total liver are removed, leaving intact the right lateral and the caudate lobes. The abdomen is closed in two layers, using 3/0 chromic catgut for the peritoneum and rectus muscle layer and 2/0 silk for the skin.

There is no special post-operative care and complications are surprisingly rare, mortality being of the order of less than 0.5%.
C. DETERMINATION OF PLASMA ALBUMIN CONCENTRATION

Reagents:

(1) 0.9% (W/V) NaCl.

(2) HC1 - Ethanol.
Prepare a mixture of 95% absolute ethanol and 5% methanol. To 600 ml of this ethanol-methanol mixture add 1.0 ml of concentrated HC1. Store at 4°C.

(3) 0.2M Sodium acetate-ethanol.
Dissolve 2.7218 mg sodium acetate in 5.0 ml methanol.
Add 95 ml absolute ethanol. Store at 4°C.

(4) 3% (W/V) sodium hydroxide.

(5) Biruet reagent.
Dissolve (i) 17.3 g copper sulphate pentahydrate in 100 ml hot distilled water.
(ii) 173 g sodium citrate and 100 g anhydrous sodium carbonate in 800 ml distilled water while heating.
When cool, pour (ii) into (i) with rapid stirring
Dilute to 1 litre with distilled water.

(6) Protein Standard.
30 mg/ml aqueous solution of crystalline human albumin.

Method:

(a) To 0.2 ml of plasma add 0.8 ml of 0.9% (W/V) sodium chloride.

(b) Add 9.0 ml HC1-ethanol. Stopper and mix by inversion.

(c) Incubate for 30 minutes at 37°C.

(d) Centrifuge.
(e) Transfer 5.0 ml of the supernatant to another centrifuge tube.

(f) Add 0.5 ml of 0.2M acetate-ethanol. Stopper and mix by inversion.

(g) Allow to stand at room temperature for 10 minutes.

(h) Centrifuge. Pour off supernatant. Invert tubes on filter paper to ensure that all supernatant fluid is removed.

(i) Dissolve precipitate in 5.0 ml 3% sodium hydroxide, add 1.0 ml biuret reagent, and mix. To one of the triplicated samples, omit biuret reagent, add 1.5 ml 3% NaOH, in order to measure turbidity.

(j) Read intensity of colour produced after 15 minutes using a 54 filter on a Klett colorimeter.

A reagent blank consists of 5.0 ml 3% sodium hydroxide plus 1.0 ml biuret reagent. The known protein standard is made by adding 4.9 ml of 3% sodium hydroxide and 1.0 ml biuret reagent to 0.1 ml of 3 g% human albumin solution.
D. AMINO ACIDS

The following quantities of amino acids were added to the perfusate in the in vitro experiments or administered to the rats in the in vivo experiments. (10 times normal peripheral blood concentration, as defined by Kirsch et al (1968b).

Arginine 19.3 mg
Asparagine 14.4 mg
Iso-leucine 6.68 mg
Leucine 12.91 mg
Lysine 29.21 mg
Methionine 5.17 mg
Phenylalanine 7.75 mg
Proline 31.63 mg
Threonine 16.52 mg
Tryptophan 3.99 mg
Valine 13.07 mg

(1) IN VITRO:

The 11 amino acids, each weighed out separately for every experiment and dissolved in 5 ml Plasmalyte B, were added to the perfusate which for these experiments was made up with 20 instead of 25 ml Plasmalyte B.

(2) IN VIVO:

The 11 amino acids, each weighed out separately for every experiment, were dissolved in 5 ml distilled water and administered to the rat immediately post-operatively by means of a naso-gastric tube.
The amino acids were administered again, in the same way, one hour before the intravenous injection of $^{14}$C-carbonate and $^{125}$I albumin.
E. MEASUREMENT OF ALBUMIN SYNTHESIS:

Reagents:

(1) 10% (W/V) and 5% (W/V) trichloroacetic acid.

(2) Absolute ethanol.

(3) CO₂-free 1N sodium hydroxide.
   (i) Prepare CO₂-free water by boiling 200 ml distilled water to half its volume.
   (ii) To 10.0 ml of 1N sodium hydroxide add 90 ml CO₂-free water.
        Stopper tightly and prepare a fresh solution for each batch of samples processed.

(4) 4 M citric acid.
    Make 84 g citric acid up to 100 ml volume with distilled water.

(5) Phosphate buffer (pH 6.7).
    Dissolve 41.75 g sodium pyrophosphate and 5.7 ml orthophosphoric acid in 500 ml distilled water.

(6) Urease solution.
    A 1 mg/ml solution of urease (Nutritional Biochemical Corporation, Cleveland, Ohio, U.S.A.) in phosphate buffer. Store at 4 °C and renew monthly.

(7) 10% (W/V) sodium tungstate.

(8) Tungstic acid.
    Make up 10.0 ml 10% (W/V) sodium tungstate and 3.8 ml 1N HCl to 100 ml with distilled water.
(9) **Citric-tungstic acid solution.**
A 1 : 1 mixture of 4 M citric acid and tungstic acid.

(10) **0.66 N sulphuric acid.**

(11) **Resins:** (Deacidite FF resin is obtained commercially in the chloride form - 100-200 mesh; 3.5% cross linkage).

(a) **Hydroxide form**

(i) Wash the chloride form with distilled water until eluate is clear.

(ii) Wash with 4 litres of 4 N NaOH.

(iii) Wash with 4 litres of 2 N NaOH.

(iv) Determine if eluate is free of chloride ions by testing with silver nitrate at acid pH.

(v) If necessary repeat washings with 4 N and 2 N NaOH until eluate is chloride free.

(vi) Wash with distilled water to pH 7.0.

(b) **Carbonate form**

(i) Wash the hydroxide form with two litres of 0.5 N sodium bicarbonate.

(ii) Wash with distilled water to pH 7.0.

(12) **Activated arginase solution.**
Prepare manganese/maleic buffer.

(i) Dissolve 8.90 g sodium maleate in 200 ml distilled water.

(ii) Bring pH to 9.7 - 9.8 with 1 N NaOH.
(iii) Add 11.5 g manganese sulphate \((\text{MnSO}_4 \cdot 4\text{H}_2\text{O})\) and make up to 500 ml with distilled water.

(iv) Bring to pH 7.0 with 1 N HCl.

Arginase solution is prepared as a 1 mg/ml solution of arginase (Nutritional Biochemical Corporation) in manganese/maleic buffer.

Add required amount of arginase to the appropriate volume of buffer, and incubate for 3 hours at 37° C. The solution is stored at 4° C and renewed every 2 months.

(13) Phenylethylamine-methanol.

(i) Re-distill commercially obtained phenylethylamine until yellow colour is removed.

(ii) Mix with an equal volume of methanol.

(iii) Store in the dark.

(14) Scintillation mixture.

Dissolve 7.5 g 2,5-diphenyloxazole (PPO) and 0.75 g 1,4-bis-(4-methyl-5-phenyloxazole-2-yl) benzene (POPOP) in 2.5 litres anhydrous toluene.

Method

(a) Preparation of samples for measurement of albumin guanidine carbon specific activity.

(1) Extraction of albumin from plasma.

(i) To 1 ml of plasma add 1 ml of bovine albumin (100 mg/ml British Drug House Chemicals Limited, Poole, England) and add 2 ml of 10% trichloroacetic acid.

(ii) Mix well, centrifuge and discard supernatant.
(iii) Wash precipitate with an equal volume of cold 5% trichloroacetic acid. Resuspend. Centrifuge and discard supernatant.

(iv) Add 3 volumes of absolute ethanol to precipitate Mix thoroughly and centrifuge to remove globulin precipitate.

(v) Dialyse albumin-rich supernatant against distilled water overnight at 4 °C.

(vi) Concentrate sample to approximately 2 ml. Confirm homogeneity of fraction by cellulose acetate electrophoresis. Measure the volume and concentration by the biuret method to give the total amount of albumin present.

(2) Acid hydrolysis of albumin

(i) Given that 100 ml of 6 N HC1 is added to 250 mg protein determine amount of 6 N HC1 required for sample (1 vi).

(ii) Add volumes of water and concentrated HC1 to sample so that the final solution is 6 N with respect to HC1.

(iii) Transfer to hydrolysis tubes and hydrolyse for 16 hours at 110-115 °C.

(iv) Transfer the hydrolysate to a round-bottom flask and evaporate to dryness.

(v) Wash dried sample in flask with 3-5 ml distilled water and evaporate to dryness again.
(3) Neutralisation of hydrolysate, and incubation with arginase.

(i) Dissolve amino acid residue in 2-3 ml distilled water.

(ii) Bring to pH 7.0 by the addition of Deacidite FF resin in the carbonate form.

(iii) Pass through a glass column containing approximately 5 mm of the carbonate resin.

(iv) Wash the flask with about 10 ml distilled water and add washings to the column.

(v) Collect eluate in a round-bottom flask.
   (a) add 2 ml activated arginase solution.
   (b) bring to pH 9.0 with 1 N NaOH.
   (c) incubate for 16 hours at 38 °C.

After incubation

(vi) Bring sample to pH 2.0 with 4 M citric acid.

(vii) Evaporate to dryness.

(viii) Dissolve this urea in 1 ml of distilled water and 0.5 ml phosphate buffer. Bring to pH 7.0 with CO₂-free 1 N NaOH.

Sample is now ready for the gas-train.

(b) Preparation of samples for measurement of urea carbon 5A

(i) In vivo

Thaw serial samples of blood in stable urea and precipitate proteins as in (ii) below.
In vitro (Perfusion study)
To 0.5 ml plasma from the sample taken at the end of the perfusion, add 2.0 ml of stable urea (1 mg/ml) - in triplicate.

(ii) Precipitate proteins by adding 1.0 ml of 10% sodium tungstate, and 1.0 ml 0.66 N sulphuric acid.

(iii) Mix well by inversion and leave to stand for 5 minutes. Centrifuge.

(iv) Place protein-free supernatant in round-bottom flask and evaporate to dryness.

(v) Redissolve in 1.0 ml distilled water and 0.5 ml phosphate buffer.

(vi) Bring to pH 7.0 with CO₂-free 1 N NaOH.
Sample is now ready for gas-train.

(c) Measurement of $^{14}$CO₂ Specific Activity (See Fig. 19)

(i) Place sample in centre bulb of reaction vessel (a)
Place 1 ml urease in one side-arm and 1 ml citric-tungstic acid solution in the other side arm.

(ii) Briefly immerse vessel in a solid CO₂-ethanol mixture and evacuate gases present in solution while gently thawing.
Seal vessel by closing tap.

(iii) Tip in urease from side-arm and allow to incubate for one hour at room temperature.

(iv) Tip acid mixture into reaction vessel and shake gently to release CO₂.

(v) Place reaction vessel on gas-train and immerse in solid CO₂-ethanol

(vi) Evacuate gas line.
Figure 19. The high vacuum gas train.
(vii) Immerse cold finger (b) in liquid nitrogen and allow CO$_2$ to pass from reaction vessel to cold finger.

(viii) Evacuate any contaminant gases present while the CO$_2$ sample is trapped under liquid nitrogen in the cold finger.

(ix) Close tap above cold finger. Thaw cold finger in warm water and then immerse in solid CO$_2$-ethanol, releasing the CO$_2$ sample but trapping any contaminant gases.

(x) Fill cuff (c) surrounding manometer arm with liquid nitrogen and allow CO$_2$ sample to pass from cold finger to manometer arm (previously evacuated) by opening the intervening taps and closing the pump tap.

(xi) Raise mercury column to trap CO$_2$ sample in the manometer arm and thaw by adding warm water to the cuff.

(xii) Adjust mercury column to the reference mark on the modified MacLeod chamber (d) to obtain the displacement of mercury by CO$_2$.

(xiii) Note the scale reading indicated by the height of the mercury column in the manometer side-arm.

(xiv) Evacuate the rest of the gas-train including the tube (e) containing 2 ml of phenylethylamine-methanol mixture frozen under liquid nitrogen.
(xv) Close the pump tap, lower the mercury and transfer the CO$_2$ to the prepared tube (xiv).

(xvi) Remove the tube and after thawing transfer the solution to a counting vial containing 8 ml of the PPO-POPOP scintillation mixture. Count each sample for two 20 minute periods in a Beckman Liquid Scintillation Counter.

(d) **Calculation of albumin synthesis rate.**

**In Vivo:**

(1) **Urea carbon specific activity measurements.**

   (i) Convert volumes of CO$_2$ into mg C by means of a standard graph.

   (ii) Calculate SA (Expressed as counts per minute/mg C) for each of urea samples.

   (iii) Correct these SA measurements for the stable urea added. 

   (iv) Plot corrected SA measurement on semilog graph paper against time.

   (v) By the least mean squares method plot the best fitting line through these points.

   (vi) Extrapolate the line back to the Y axis and determine urea SA at $t_0$.

   (vii) From the slope determine the fractional SR of urea per hour.

+ Equal recovery of the stable and labelled urea occurs.
(2) **Albumin guanidine carbon specific activity measurement.**

(i) Calculate albumin SA as in (i) and (ii) above.

(ii) Correct the SA measurement for the added bovine albumin *.

(iii) Determine albumin SA at $t_0$ by multiplying the observed SA at $t_0$ by the factor derived from the disappearance of the iodinated albumin sample over the 6 hour experimental period.

Then:

Fractional SR of albumin

= \frac{\text{Fractional SR urea} \times 24 \times \text{albumin SA at } t_0}{\text{Urea SA at } t_0}

Absolute SR albumin

\text{(mg/kg/24 hours)}

= \frac{\text{FSR albumin} \times \text{intra-vascular albumin pool (mg)}}{\text{Weight in Kg} \times 100}

**In Vitro (Perfusion study)**

(1) **Urea, carbon radioactivity**

(i), (ii) and (iii) as before

(iv) Calculate the urea pool in mg C.

Urea pool in mg C

= \frac{\text{Perfusate volume (ml) \times final urea concentration (mg\%)} }{500}

Where $\frac{12}{60} = \text{factor for converting mg urea to mg C in urea}$

* Equal recovery of the stable and labelled albumin occurs.
(v) Calculate the total radioactivity incorporated into urea.

Total radioactivity incorporated into urea (counts/min)

\[ = \text{urea pool} \times \text{urea SA} \]

\[ \text{(mg C)} \quad \text{(C/min/mgC)} \]

(2) Arginine carbon radioactivity

(i) and (ii) as before.

(iii) Calculate the arginine pool in mg C.

Arginine pool

\[ = (100-\text{PCV}) \times \text{perfusate volume} \times \text{perfusate albumin concentration} \times 5.95 \times 12 \]

\[ = 17,400 \times 100 \]

Where

(1) \( (100-\text{PCV}) \times \text{perfusate volume} \, \text{ml} = \text{plasma volume} \, \text{ml} \)

\[ \frac{100}{100} \]

(2) PCV = Packed Cell Volume

(3) \[ 5.95 \times 12 = \text{factor for converting mg albumin to mg guanidine carbon or arginine in albumin} \]

\[ = 174 \times 100 \]

(4) Albumin concentration expressed in mg/ml.

(iv) Calculate the total radioactivity incorporated into arginine.

Total radioactivity incorporated into arginine (counts/min)

\[ = \text{arginine pool} \times \text{arginine SA} \]

\[ \text{(mg/C)} \quad \text{(C/min/mg/C)} \]

(v) Calculate urea synthesis rate.

Urea synthesis rate (mg/hr)

\[ = \text{perfusate volume} \times \text{(post-pre urea concentration in mg/ml)} \]
(vi) Calculate the arginine synthesis rate.

\[
\text{Arginine synthesis rate (mg/hr)} = \frac{\text{Radioactivity incorporated into arginine}}{\text{Urea synthesis rate}} \times \text{Radioactivity incorporated into urea}
\]

(vii) Calculate the albumin synthesis rate.

\[
\text{Albumin synthesis rate (mg/hr)} = \frac{\text{Arginine synthesis rate} \times 100}{5.95}
\]

Where 5.95 = amount of arginine in albumin

(viii) Correct the albumin synthesis rate for body weight.

\[
\text{Corrected albumin synthesis rate (mg/hr/300 g)} = \frac{\text{Albumin synthesis rate} \times 300}{R}
\]

Where \( R \) = rat body weight.
F. DETERMINATION OF UREA CONCENTRATION (Nessler's method)

Reagents:

(1) Urease 1 mg/ml solution.
(2) Iodine - stock solution. 2 gm I₂ + 3 gm KI up to 100 ml water. Working solution - dilute 1 in 100.
(3) Nessler's reagent
   (a) Add 400 ml distilled water to 100 mg Mercuric Iodide + 70 gm KI.
   (b) Dissolve 150 gm NaOH in 500 ml distilled water. Cool thoroughly and add to (a) with constant shaking. Make up to one litre with distilled water and keep in dark cupboard until reddish precipitate settles out. Now ready for use.

Method: (in triplicate)

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard (50 mg/ml urea)</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>2.2 ml</td>
<td>2.0 ml</td>
<td>2.0 ml</td>
</tr>
<tr>
<td>Serum</td>
<td>-</td>
<td>-</td>
<td>0.2 ml</td>
</tr>
<tr>
<td>Urease</td>
<td>0.2 ml</td>
<td>0.2 ml</td>
<td>0.2 ml</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>0.2 ml</td>
<td>-</td>
</tr>
</tbody>
</table>

Incubate at 37°C for 3/4 hour

0.66N H₂SO₄ 0.3 ml 0.3 ml 0.3 ml
10% sodium tungstate 0.3 ml 0.3 ml 0.3 ml
Distilled water 5.0 ml 5.0 ml 5.0 ml

Mix and centrifuge at 3000 rpm in bench centrifuge for 20 minutes. To 5 ml supernatant from each tube add 4.5 ml distilled water and 0.5 ml working solution of Iodine. Immediately before reading add 1 ml Nessler's reagent to each tube.

Read on Pye Unicam Spectrometer at 480 nm.
G. PREPARATION OF ALBUMIN BY POLYETHYLENE-GLYCOL METHOD

Reagents:
(1) Phosphate buffer pH 8.6, 0.067 molar containing disodium ethylenediaminetetraacetate (EDTA) 0.01 molar.
(2) Pulverized Polyethylene Glycol (PEG).
(3) Pulverized Potassium Dihydrogen Phosphate (KH₂PO₄).
(4) Chloroform.

Method
(1) 1 part serum plus 2 parts phosphate buffer.
(2) Add 30% PEG.
(3) Centrifuge at 10,000 g in 25 rotor in ultracentrifuge for 30 minutes.
(4) Pipette off supernatant fluid.
(5) To supernatant fluid add KH₂PO₄ slowly, while stirring, to pH 5.6.
(6) Stand on bench for 30 minutes.
(7) Spin at 10,000 g for 30 minutes in 25 rotor in ultracentrifuge.
(8) Pipette off albumin layer - bottom phase.
(9) Dilute five times with pH 8.6 buffer.
(10) Add 2 volumes chloroform and shake.
(11) Spin at 4,000 rpm in Sorvall high-speed centrifuge for 30 minutes.
(12) Remove top layer. Dry off chloroform.
(13) Dialyse against distilled water overnight.
(14) Test for purity by electrophoresis.
H. IODINATION

Reagents

(1) Approximately 15 mg rat albumin (prepared as in F) contained in 0.2 ml volume is used for labelling.

(2) Iodine monochloride (stock solution).
   (i) Dissolve 150 mg sodium iodide in 8.0 ml 6N HCl.
   (ii) Dissolve 108 mg sodium iodate monohydrate in 2.0 ml distilled water.
   (iii) Forcibly inject (ii) into (i) to avoid precipitation of iodine.
   (iv) Dilute to 40.0 ml with distilled water and shake with 5.0 ml carbon tetrachloride. The presence of a red discolouration in the organic solvent indicates the presence of free iodine. The carbon tetrachloride is removed with a pipette, and the extraction process is repeated until the carbon tetrachloride remains unchanged in colour.
   (v) Bubble moist air through ICl solution for one hour to remove any residual carbon tetrachloride.
   (vi) Make up to 45.0 ml with distilled water. This solution is stored at 4°C.
   (vii) Before use, dilute 1 volume of stock solution with 9 volumes of 2M NaCl.

(3) Glycine buffer (stock solution)
   (i) Dissolve 7.5 g glycine in 75.0 ml distilled water.
   (ii) Add 25.0 ml 1N NaCl.

(4) Alkaline glycine buffer (pH 9.0 - 9.5)
   Add 0.2 ml 1N (W/V) NaOH to 1.8 ml of the stock solution.
Method

(a) Prepare alkaline glycine buffer as above.
(b) Prepare 1.0 ml of a solution of IC1 containing 0.1 ml IC1 with 0.9 ml 2M NaCl.
(c) Place required amount of radiiodine solution in 0.3 ml of the IC1 prepared in (b). (Tube A).
(d) Adjust pH of albumin solution to 8.5 with alkaline glycine buffer (Tube B).
(e) Similarly, bring pH of Tube A to 8.5.
(f) Without delay vigorously and only once inject contents of Tube A into Tube B.
(g) Remove the free iodine by dialysis against distilled water.
(h) Check the free radioiodine content on an aliquot of the final preparation by trichloroacetic acid precipitation to ensure less than 1.0%.
(i) Check that the label is confined only to the albumin fraction by cellulose acetate electrophoresis.
(j) Sterilize by Seitz filtration.
I. DETERMINATION OF UREA CONCENTRATION (Diacetyl Monoxime Method.)

Reagents:

(1) Trichloracetic acid (TCA) - 20%

(2) Diacetyl monoxime solution

Diacetyl monoxime 10 gms
Sodium chloride 300 gms
Distilled water 2 litres

(3) Ferric alum acid Reagent:

Ferric alum (FeNH₄(SO₄)₂ 12H₂O) 5.0 gm
Add distilled water 500 ml
Cool, add Orthophosphoric acid (concentration 85%) 500 ml
Mix, add sulphuric acid (concentrated) 500 ml
and filter.

Technique:

In centrifuge tubes prepare protein filtrates:

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Test</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>1.0 ml</td>
<td>0.8 ml</td>
<td>0.8 ml</td>
</tr>
<tr>
<td>Serum</td>
<td>-</td>
<td>0.2 ml</td>
<td>-</td>
</tr>
<tr>
<td>Standard urea</td>
<td>-</td>
<td>-</td>
<td>0.2 ml</td>
</tr>
<tr>
<td>solution</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20% TCA</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
</tbody>
</table>

Whirlimix: centrifuge for 10 minutes at 3,000 rpm. Filter supernatant fluid through cotton-wool pledgets.
(2) In quick fit tubes:

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Test</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prepared filtrates</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>Diacetyl monoxime</td>
<td>4.0 ml</td>
<td>4.0 ml</td>
<td>4.0 ml</td>
</tr>
</tbody>
</table>

Shake all tubes thoroughly. Add 4.0 ml ferric alum-acid reagent to each tube, one at a time, shake and stopper and place in a boiling water bath at one minute intervals. Boil for a fixed period (10-15 minutes) and remove in succession at one minute intervals. Cool in a beaker of tap water. After 5 minutes read tubes in succession of one minute intervals on Pye Unicam Spectrometer at 470 nm.
J. MEASUREMENT OF ALBUMIN CATABOLISM

(1) Assay of samples:
   (i) Deep frozen serial serum samples thawed.
   (ii) 10\alpha of (i) diluted in 3 ml distilled water.
   (iii) Radioactivity assayed in a Packard Autogamma Scintillation Spectrometer (Model 578).

(2) Plasma albumin concentration of the final sample was determined by the Fernandez method (see Appendix C p. 57).

(3) Calculation of Results
   (i) Take immediate post-injection whole-body counts and those of the 15 minute plasma sample to represent 100\% of the administered dose. After correction for appropriate standards, plot successive daily whole-body and plasma counts as a percentage of the dose on semilog graph paper.
   (ii) Derive best-fitting curves, using least mean squares method.
   (iii) From the graphs, calculate daily urinary loss (equivalent to fall in whole-body activity) and midpoint of plasma activity for the corresponding 24-hour period.
   (iv) Determine daily fractional catabolic rate (FCR) from
       \[
       \text{Albumin catabolic rate (mg/24 hr)} = \frac{\text{Urinary excretion in 24-hours}}{\text{Mean plasma activity}} \times 100\%
       \]
   (v) Calculate absolute values for catabolic rates from fractional rates and plasma albumin pools.
(vi) Correct the albumin catabolic rate for body weight

Corrected albumin synthesis rate (mg/24 hr/300g)

= \frac{\text{albumin catabolic rate} \times 300}{R}

Where R = rat body weight.
K. **STATISTICAL METHODS**

(1) **Mean**

\[ \bar{x} = \frac{\sum x}{n} \]

Standard deviation of the mean. \( S = \frac{\sum (x - \bar{x})^2}{n - 1} \)

(2) **Student's t-test**

\[ t = \frac{\bar{x}_1 - \bar{x}_2}{S \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \]

with \( n_1 + n_2 - 2 \) degrees of freedom

(3) **Linear regression analysis (Method of Least Mean Squares)**

The least square line approximating the set of points \((x_1, y_1), (x_2, y_2), \ldots, (x_n, y_n)\), is given by the equation

\[ y = mx + c \]

where

\[ m = \frac{\sum xy - (\sum x)(\sum y)}{\sum x^2 - (\sum x)^2} \]

and

\[ c = \frac{(\sum y)(\sum x^2) - (\sum x)(\sum xy)}{\sum x^2 - (\sum x)^2} \]

(4) **Wilcoxon test for two samples**

Given two samples 1 and 2 with values \( x_1 \) and \( x_2 \) with means \( \mu_1 \) and \( \mu_2 \). The values are ranked as a whole. The sum of the rank numbers for sample 1 = \( T_1 \). Similarly those for sample 2 = \( T_2 \).

Significance limits for \( T_1 \) are given in Documenta Geigy Scientific Tables P. 124, (Edited by Konrad Diem, published by J.R. Geigy, S.A., Basle, Switzerland. Sixth edition) which list probability

\[ T_{\alpha} < T < T_{\beta} \]

If \( T_1 \) attains the significance levels or exceeds them in the outward direction from its expected value, then this may be regarded as evidence that:
\[ u_1 < u_2 \text{ if } T_1 \leq T_e (\leq a) \]
\[ u_1 > u_2 \text{ if } T_1 \geq T_r (\leq a) \]
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