FACTORS AFFECTING TISSUE GROWTH

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THESIS SUBMITTED TO THE UNIVERSITY OF GLASGOW
FOR THE DEGREE OF MASTER OF SCIENCE

OCTOBER 1971

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789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

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A study has been undertaken of various factors which affect the growth of tissue. It has been known for over a century that, when one of the kidneys of a laboratory animal or of a human being is diseased or has been surgically removed, its partner undergoes compensatory growth. This phenomenon is termed compensatory renal hypertrophy and has been used as the basic experimental model in the present investigation. The index of growth used in these studies was sometimes total renal weight, or total renal weight relative to body weight and on other occasions kidney composition was estimated by making use of the fact that in the kidney the DNA content per cell is constant. An estimation of the DNA content of the kidney, therefore, will give an indication of the number of cells present, and by relating other cellular constituents to DNA, an indication can be obtained of the average cell composition. Some experiments were also performed in vitro using adult rat kidney cells aggregating on micropore filters and forming a biochemically viable colony; in these experiments growth was estimated by assay of the incorporation of radioactive isotope into DNA, RNA and protein.

It has been shown that the correlation between total renal weight and rat body weight is not particularly good, whereas that between liver weight and rat body weight is good; the correlation between

the dry weight of the kidneys and rat body weight is fairly good as is that between the dry weight of the liver and body weight. The best correlation between the various parameters is that between the dry weight of the kidneys and the weight of the liver.

In seeking the stimulus to the compensatory growth which occurs after removal of one kidney, changes in the physiological and biochemical state of the rat have been investigated after unilateral nephrectomy. In fact, very little change occurs in the more important physiological and biochemical parameters following unilateral nephrectomy. There is no alteration in plasma sodium levels in the first 48 hours after unilateral nephrectomy in the rat when compared to sham operated animals. Both nephrectomized and sham operated rats have an elevated pH 16 hours after unilateral nephrectomy; the indications are that this occurs as a result of hyperventilation causing loss of CO_2. Plasma amino acid analysis has shown a reversal of the normal glutamine/glutamate ratio with a marked fall in the plasma glutamine level and a corresponding increase in the plasma glutamate level; this is present only at 14 hours after unilateral nephrectomy. There is no consistent alteration in the blood ammonia level in the first 48 hours after unilateral nephrectomy. There is no significant increase in the amidotransferase activity of the remaining kidney 48 hours after unilateral nephrectomy. However, there is a suspicion that sham operation depresses the level of kidney amidotransferase activity. Unilateral nephrectomy causes no

immediate alteration in the level of arterial blood pressure. Thus, there is little indication of what might be the stimulus to compensatory renal hypertrophy.

Growth of the kidney might theoretically be modified by various dietary and parenteral means. It has been shown that the repeated intravenous injection of a urea/saline mixture has no effect on renal weight or composition in the otherwise intact rat and produces no alteration in the normal growth response of the kidney remaining after unilateral nephrectomy. The administration of excess quantities of sodium chloride or potassium chloride in the diet does not increase kidney weight in the rat. The administration of a diet high in the protein gelatin produces an increase in renal weight in the rat and in addition increases the RNA and protein contents of the kidney. The changes in kidney composition are similar to those which occur in the kidney remaining after unilateral nephrectomy but are not identical since gelatin does not produce the increase in RNA/100 mg or protein/100 mg which is found after unilateral nephrectomy but does produce a marked increase in mean cell mass; this suggests the accumulation of some other substance within the cell, perhaps water. A diet containing a quantity of glycine equivalent to that which is contained in the gelatin-supplemented diet causes an increase in kidney weight of much smaller order than that produced by gelatin; analysis of the kidneys shows an increase in RNA and, interestingly enough, an increase in mean cell mass, i.e. the growth is of the same type as

that produced by gelatin and seems once more to be of a different type to that which occurs in the kidney remaining after unilateral nephrectomy. The amino acid arginine HCl causes an increase in kidney weight and a marked fall in body weight and a fall in liver weight proportionally greater than the fall in body weight; the amino acid arginine-free base causes no increase in kidney weight but produces an increase in the kidney weight/body weight ratio. Presumably arginine is toxic to the rat in large quantities; the increase in renal weight found after feeding arginine HCl must be due to a metabolic acidosis induced by HCl.

The administration of sodium and potassium citrate also affects kidney weight and composition. Potassium citrate gives an actual increase in renal weight as well as a relative increase, i.e. it also increases the renal weight/body weight ratio; it shows an interaction with glycine in reducing renal RNA content and in increasing the RNA/DNA ratio. It would appear that the reduction in RNA caused by a diet high in potassium citrate is due to its ability to act as an alkalinizing agent whereas the increase in the RNA/DNA ratio in the kidneys of rats fed a diet high in glycine as well as potassium citrate suggests the interplay of some other factor, perhaps overhydration of the cell. The reduction in body weight found after potassium citrate administration seems to be due to the potassium ions since sodium citrate has no effect on rat body weight but shows the same effect as potassium citrate on kidney weight when fed along with glycine.

Sodium acetate causes an increase in renal weight which is of the same order as the increase in the renal weight/body weight ratio whereas potassium acetate causes only an increase in the renal weight/body weight ratio, i.e. potassium acetate causes a greater fall in body weight than potassium citrate. Potassium citrate has no effect on the kidney lipid content. An increase in the glucuneogenic activity of the rat produced by feeding a high fat diet has been shown to cause no increase in kidney weight or liver weight. However, a high fat protein free diet causes an increase in the liver weight/body weight ratio, but does not affect the kidney weight or the kidney weight/body weight ratio.

The hypothesis that compensatory renal hypertrophy is initiated by a humoral substance has been investigated by an in vitro technique involving adult rat kidney cells aggregating on micropore filters. Evidence has been found for the presence of such a substance in the blood of nephrectomized rats during the present investigation. The reason for the failure to demonstrate the presence of this factor or factors in previous in vivo experiments has also been explained; the concentration of plasma required to produce a positive result is such as could not be obtained by transfer from one animal to another.

In addition, 2 experimental models previously found to increase the growth of the kidney remaining after unilateral nephrectomy have

been re-investigated. Simultaneous partial hepatectomy has no effect on compensatory renal hypertrophy in the rat; also, simultaneous unilateral nephrectomy has no effect on the rate of growth of the liver fragment remaining after partial hepatectomy in the rat. This disproves the presence of general growth promotional agents in the blood. Simultaneous renal denervation and contralateral nephrectomy has been shown to produce profound changes in the physiological state of the rabbit, causing hypernatraemia, hypocalcaemia, a fall in PCV and acute renal nephrocalcinosis. These changes invalidate the use of this experimental model in the study of compensatory renal hypertrophy.

Future work should be directed towards isolating and characterising the humoral agent, towards studying the earliest changes in RNA metabolism in the kidney remaining after unilateral nephrectomy and during the administration of gelatin, glycine, citrate and acetate salts and towards investigating the possible effects on cell and body growth of variations in the cellular and extracellular concentrations of sodium, potassium and water.

Reference

Malt, R. A. (1969) : New Eng. J. Med. 280, 1446.

ACKNOWLEDGEMENTS

I wish to thank Professor J. N. Davidson and Professor R.M.S. Smellie for the opportunity to work in the Department of Biochemistry between 1967 and 1969, and Professor Smellie for background support and encouragement during the performance of this work and preparation of this thesis. I am indebted to Dr. R. Imrie for an introduction to modern concepts of cell growth and control. I am also indebted to Dr. T. Anderson for help with histological preparation and Mr. D. MacAllister for photography. My gratitude is especially due to Miss Irene Breen for her infinite care in typing and to Mr. T. Allison of Rank Xerox. I thank the Faculty of Medicine for receipt of the Galloway Research Fellowship.

The Supervisor was R. Y. Thomson, B.Sc., Ph.D., Senior Lecturer in Biochemistry, at whose bench the work was performed, and who participated in the experiments in Sections 3.2.2, 3.2.6, 3.3.1, 3.5.1 and 3.5.2. Dr. Thomson and I wish to thank Dr. G. Leaf for amino acid analyses.

CONTENTS

SECTION 1

INTRODUCTION

COMPENSATORY RENAL HYPERTROPHY

1.1 : GENERAL INTRODUCTION

Total nephrectomy is followed by death within a few days; however, the removal of one kidney is perfectly compatible with a normal life span (Hinman, 1943). If one kidney is diseased to the point of atrophy, its partner shows a corresponding hypertrophy. This hypertrophy is also seen after surgical removal of a kidney; the process is called Compensatory Renal Hypertrophy and has been under investigation since it was first described by Rayer in 1841 (Braun-Mendenez, 1952).

1.2 : STRUCTURAL ASPECTS OF COMPENSATORY RENAL HYPERTROPHY

1.2.1 : Renal Mass

Many observations of the increase in renal mass following unilateral nephrectomy have been made and the most important of these are shown in Table 1. Despite inevitable biological variation, there appears to be general agreement about the rate of hypertrophy. In the rat - the most studied species - there is a true increase in dry mass of the order of 3% after 24 hours. Reported increases of wet mass of 17% after one day in the rat and 10 - 20% after two hours in the rabbit are rather more than average, and changes in wet weight generally parallel changes in dry weight. After the first day growth is rapid, 30 - 40% at the end of one week, 50% at 17 - 40 days,

TABLE 1

Investigator(s)	Date	Species	Body Weight (g)	Observed Weight Increase in the Remaining Kidney
Kurnick	1955	Rat	160-240	3% at 1 day 40% at 18 days
Becker and Ogawa	1959	Rat	180	11% at 3 days 35% at 10 days 44% at 17 days
Montfort and Perez-Tamayo	1962	Rat	79-159	22% at 10 days 39% at 20 days
Addis and Lew	1940	Rat	None given	70% at 40 days
Strauber and Patt	1961	Mouse	Various	40% at 14 days 50% at 26-36 days
Fajers	1957	Rabbit	1500-2800	Significant at 1 day 20% at 10-20 days
Addis, Myers and Oliver	1924	Rabbit	None given	58% at 15-33 days 66% at 106-126 days

The effect of unilateral nephrectomy on the weight of the remaining kidney.

and 70% in the longest intervals included in experimental studies. Similar results have been obtained in mice and rabbits, with smaller increments in the dog.

1.2.2 : Growth of the Nephron : Histology

No new nephrons are formed during compensatory growth even if unilateral nephrectomy is carried out in the neonatal period. The diameter of the glomeruli doubles or triples, but no new glomeruli are formed. Hiramoto, Bernecky and Jurand (1962) have shown that glomeruli uniformly labelled with anti-rat kidney serum show no dilution with unlabelled glomeruli even after seven months in the renoprival state. Compensatory growth consequently consists of growth of the nephrons already present.

1.2.3 : Mitotic Index

Most growth of the tubules is accounted for by hypertrophy, i.e. increase in cell size, but hyperplasia, i.e. increase in cell number, also occurs. Johnson and Vera Roman (1966) report that in the rat at five days after unilateral nephrectomy a 7% increase in renal cell number accounts for 25% of the growth, hypertrophy accounting for the remaining 75%.

The rate of cell division in the normal adult rat kidney is very low. Franck (1960) reports it as 15 to 40 per 100,000 and Goss and Rankin (1960) as 24 per 100,000. McCreight and Sulkin (1959) report the mitotic frequency as 100 per 100,000 in the proximal tubule of young adult rats, falling to 25 per 100,000 in senile rats.

Argyris and Trimble (1964), using colchicine to arrest mitoses in mice kidney, found a mitotic frequency twice as high in males as in females. In the normal kidney the mitotic rate is known to vary with the time of the day. Blumenfeld (1942) and Williams (1961) both place the maximum at between 2 and 4 p.m. but, whereas Blumenfeld places the minimum at 10 - 12 p.m., Williams (1961) puts it at 6 - 8 a.m. Zakharov (1961) has also found mitotic frequency high in the morning and low at night. This type of diurnal variation is also found in other organs, notably the liver (Jaffe, 1954). Mitoses are most numerous in the proximal tubules, least numerous in the distal tubule and collecting ducts and intermediate in the ascending limbs of the loops of Henle (Williams, 1961).

Following nephrectomy there is an increase in mitotic activity, generally in the rat in a burst occurring on the second day (Rollason, 1949). Some investigators note two peaks of mitotic activity. Ogawa and Sinclair (1958) and Franck (1960) report one at two days and another on the seventh day. Sulkin (1949) reports two peaks, one at three days and the second at ten days. Williams (1961) reports the primary mitotic response at 40 hours, with a smaller secondary response at 3 - 4 days.

The magnitude of the response is not great. Franck (1960) and Goss and Rankin (1960) report the mitotic rate as 3 - 6 times normal. Argyris and Trimble (1964), using colchicine to arrest mitoses in mice, found a sex difference, the increase being elevenfold in males

and fivefold in females. McCreight and Sulkin (1959) investigated age differences and report a sevenfold increase in young rats and five- to sixfold in senile rats. Bugge-Asperheim and Kiel (1968a) report an eightfold increase in the dog and a fourfold rise has been reported in hamsters by Reiter (1968).

The distribution of the mitotic increase follows the same pattern as in the normal kidney. It is highest in the cortex, particularly the tubular cells (Carnot and May, 1938; Ogawa and Sinclair, 1958). The outer medulla responds to a lesser extent and the inner medulla hardly at all.

1.2.4 Fine Structure

In the rat cytoplasmic basophilia intensifies within hours and soon after the number of free ribosomes and small vesicles in the proximal tubule increases. The cisternae of the rough endoplasmic reticulum dilate, the Golgi membranes and the agranular reticulum proliferate and at 96 hours there is a further increase in the number of cytoplasmic ribosomes. In the cortical portion of the collecting duct a less differentiated type of cell appears at 72 hours. In the distal tubule de-differentiation does not occur, but besides that the pattern is much the same (Anderson, 1967).

Leak and Rosen (1966) have done a similar ultramicroscopic study in the mouse. The main feature reported by them is the appearance of many whorls of membranous structures in the medial and basal portions of the proximal tubular cells at 48 hours. Malt

(1969) suggests that these may be present as early as 30 minutes after unilateral nephrectomy. These structures have no ribosomes attached to them and the investigators report no increase in the number of ribosomes attached to pre-existing membranes.

1.3 CHEMICAL ASPECTS OF COMPENSATORY RENAL HYPERTROPHY

1.3.1 DNA Synthesis

The DNA content per set of chromosomes is constant in the somatic cells of different tissues of any given species (Vendrely, 1955). Kidney cell nuclei all have about the same DNA content and therefore are presumably all diploid. The increase in cell number occurring after unilateral nephrectomy is paralled by an increase in total renal DNA. Mandel, Mandel and Jacob (1950a) report increases in DNA of 16% at 15 days, 18% at 30 days and 37% at 80 days. Kennedy (1960) reports a 15% rise at 21 days and 58% at 42 weeks. Threlfall, Cairnie, Taylor and Buck (1964) found a 21% increase at 21 days. Barrows, Roeder and Olewine (1962) report no variation in the response between young and old rats.

This increase in DNA is not evident immediately after the operation. Miyada and Kurnick (1960) found no increase in rat renal DNA in the first four days but found a 30% increase between the sixth and sixteenth days. Mandel, Mandel and Jacob (1950a) found a 4% increase at 7 days, and Lotspeich (1965) found a 20% increase at this time. In a recent report, Kurnick and Lindsay

(1968) found a 10% increase in DNA at 9 days and 25 - 30% at 2 weeks. Halliburton (1966) found that cell division gets under way at 48 hours after operation at which time an increase in DNA is just detectable. At 96 hours he found a 10% increase in kidney DNA. In the mouse, Malt and LeMaitre (1968) found a 14% increase in DNA at 4 weeks.

Cytophotometric evidence suggests that, when a cell is about to divide, it accumulates the additional amount of DNA beforehand (Howard, 1956). Consequently a proportion of the nuclei in a population with frequent mitoses should possess a greater than normal DNA content. Kurnick (1955) found no increase in mean DNA per nuclei using chemical analysis of nuclei isolated in bulk; however, Ogawa (1961) found a 15% increase between 3 and 5 days during compensatory renal hypertrophy, using similar methods. Franck (1958) and Becker and Ogawa (1959), making cytophotometric measurements of individual nuclei, report that a few days after operation the number of nuclei with twice the normal amount of DNA is significantly increased.

Isotope uptake has also been used to demonstrate increased DNA synthesis and the kinetics of DNA labelling agree with the histologic and chemical patterns. The specific activity of the precursor pools of the deoxynucleotides must also be measured if a true picture of DNA synthesis is to be measured. Simpson (1961), using ^{32}P, found an increase in labelling of the major deoxynucleotides of 1-2 times at 24 hours, 2-5 times at 48 hours post-operatively, falling

thereafter but showing a secondary rise at 9 days. Royce (1963) also found an increase in ^{32}P uptake 2 days post-operatively. Other workers have found roughly similar results, using ^{3}H thymidine and autoradiography. Lowenstein and Stern (1963) found an increase in labelled cells at 2 days post-operatively and also that the majority of the labelled cells were in the cortex. Reiter and McCreight (1965) report almost identical results to those of Lowenstein and Stern. Benitez and Shaka (1964) found a significant increase in the number of labelled cells at 24 hours after nephrectomy; the number fell slightly after 48 - 72 hours but still showed an increase over controls.

Noltenius, Klempermann and Oeklert (1964) found that the number of labelled cells fell in the first 2 days but rose from the third to the sixth day.

It can be seen that the chemical, cytophotometric and isotopic estimations of DNA synthesis are in agreement with the histological observations of mitotic activity seen in compensatory renal hypertrophy.

1.3.2 RNA Synthesis

In contrast to the slow and small increase in DNA during compensatory hypertrophy of the kidney, RNA shows a large and rapid increase. Mandel, Wintzerith, Jacob, Perey and Mandel (1957) report an increase in RNA before any other tissue component; the increase in RNA was 20% after 24 hours and 30 - 40% after 3 days.

This increase is consistent with the idea that the RNA is being accumulated in preparation for extensive protein synthesis, rather than merely as part of a general growth of the tissue. After seven days of compensatory growth Lotspeich (1965) reports a 37% increase in RNA. Barrows, Roeder and Olewine (1962) have investigated the effect of age of the rats on the increase in RNA and report that after 8 weeks there is a 45% increase in RNA in young adult rats but only a 34% increase in old animals. Halliburton and Thomson (1965) and Kurnick and Lindsay (1968) have studied the changes in RNA in the first few days after nephrectomy. There is a 7% increase in RNA at 12 hours, rising to a peak of 33% at two days, at which time DNA synthesis is most active. Malt and Lemaitre report the peak as being 20% in the mouse.

As has already been stated, DNA per cell nucleus is virtually constant; consequently a picture of the average composition of the cell can be obtained by comparing other constituents to DNA. Mandel et al. (1950) and Barrows et al. (1962) have shown that two months after unilateral nephrectomy the RNA/DNA ratio of the hypertrophied kidney is the same as in normal kidney, although the total RNA and total DNA have both increased. Kurnick (1955) however showed that the RNA/DNA ratio increased in the first 3-4 days and thereafter returned to the normal value on the ninth day. Halliburton and Thomson (1965) investigated mean cell composition by these methods and found an increase in RNA/DNA ratio, detectable at 12 hours, reaching 25% at 24 hours and remaining at

or above this level between 48 and 96 hours.

Malt and LeMaitre (1968) have studied not only the accretion but also the turnover of RNA during compensatory renal hypertrophy in mice. They report that the half-life of ribosomal RNA (r RNA) which makes up 85% of the RNA of the cell is 4.5 days in both normal and hypertrophying kidney. Therefore this increase in RNA must represent de novo synthesis rather than diminished breakdown of RNA. This elevated RNA synthesis is reflected in increased numbers of polyribosomes in the hypertrophied kidney, but there is no alteration in the distribution of sizes of either polyribosomes found free in cytoplasm or those bound to the endoplasmic reticulum (Malt, 1969; Priestley and Malt, 1969). Halliburton (1970) reported an increase in single ribosomes, though this work was done on the rat and different methods of preparation of polyribosomes were used than in Malt's studies.

Polysomes are the sites of protein synthesis in the cell and are made up of a number of ribosomes attached to a strand of messenger RNA (m RNA). An increase in polysomes could result from :

(a) An increased number of ribosomes binding to an unchanged amount of RNA;

(b) An increase in available binding sites;

(c) An increase in m RNA;

(d) A combination of the above.

Classes of RNA

Recently a great deal of attention has been devoted to the study of the changes in the different classes of RNA during compensatory renal hypertrophy. Since more than 85% of RNA is ribosomal and since the accretion of RNA following unilateral nephrectomy is so rapid there can be little doubt that r RNA is increased but the magnitude and time of onset of the increase is still in doubt (Malt, 1969). Halliburton (1966) found no difference in ^{3}H adenine incorporation into RNA, between sham-operated and nephrectomised animals. However, using ^{3}H orotic acid he found that the ratios of the specific activities of kidney r RNA to liver r RNA were between 25% and 120% greater in nephrectomised animals than the corresponding ratios in control sham-operated animals. These animals were rats and were sacrificed from 20 minutes to 2 hours after nephrectomy. Malt (1969) has found that in mouse kidney the labelling of nucleolar precursors of RNA with uridine is depressed immediately after either unilateral nephrectomy or sham operation. Malt and Stoddart (1966) have shown that 4 days after nephrectomy ribosomes from the remaining kidney are labelled with ^{14}C orotic acid 2 - 4 times faster than after sham operation.

However, these experiments must be interpreted with some care, and with justifiable reservations concerning the equating of rates of labelling with rates of synthesis, and the discrepancy in the above results may be due to differential incorporation of orotic acid and

uridine into kidney RNA.

Malt and Stoddart (1967) in a series of experiments in which nuclear precursors of r RNA and mature cytoplasmic RNA were compared found a cyclic alteration in r RNA synthesis in the remaining kidney, with maxima at 2 and 8 days. They showed that the increase in polysomes is in part at least due to synthesis of new ribosomes. Also, ribosomes that remain free in the cytoplasm are synthesised before membrane-bound ribosomes (Malt and Lemaitre, 1967). In the kidney, 75% of all ribosomes are free in the cytoplasm whereas in other organs such as liver or pancreas 75% of these structures are membrane-bound (Priestley and Malt, 1969). The liver and pancreas are organs which manufacture much protein for export and it is the membrane-bound ribosomes on which these are made. In the kidney, glycoproteins are manufactured on the bound ribosomes (Malt and Lemaitre, 1967); the familia of the free ribosomes is unknown. No change has been found in the proportion of free to bound ribosomes during compensatory growth (Priestley and Malt, 1969). m RNA has not been so extensively investigated mainly because it cannot be identified with certainty after extraction and it is liable to degradation.

Nuclear RNA

Cell nuclei may be fractionated into nucleoli and nucleoplasm. Willems, Musilova and Malt (Malt, 1969), using polyacrylamide gel high resolution analysis, have shown that for an hour after

laparotomy alone there is inhibition of labelling of nucleolar RNA; nucleolar RNA is the source of mature cytoplasmic r RNA. Somewhat later, after contralateral nephrectomy, there is increased labelling of precursors of r RNA.

However, within less than one hour they have found increased labelling of nucleoplasmic RNA. Nucleoplasm contains rapidly labelled heterodisperse RNA (Hn RNA), the most common type of RNA found in the nucleus (Darnell, 1968). Its function is unknown and it is not thought to be a precursor of m RNA. It has a very rapid turnover and it has been suggested that it represents a pool of non-specific components of RNA which may be incorporated into the pathway most required at a particular time.

The acceleration in Hn RNA processing begins at 10 minutes after uninephrectomy and lasts at least 7 days, and this suggests that uninephrectomy initiates immediate changes in the remaining kidney. It is not known whether Hn RNA metabolism is affected in other growing organs.

In summary therefore, uninephrectomy produces a change in RNA metabolism within the first hour. Later RNA synthesis starts and reaches a peak at 2 days, after which DNA synthesis reaches its peak and mitosis occurs.

1.3.3 Protein Synthesis

There is no doubt that protein synthesis increases in the first 24 hours of compensatory renal growth. Coe and Korty (1967)

report 2 peaks with one active phase in the first 24 hours, a slowing down between 24 and 48 hours, and a second increment between 48 and 72 hours to give a 28% increment which remains constant after 96 hours. The endogenous pool of free amino acids has been reported as decreasing in the first 24 hours after uninephrectomy and increasing between the 8th and 15th days, thereafter returning to normal. After unilateral nephrectomy the rate of incorporation of L-leucine into protein is accelerated, but that of L-glutamine is not (Bignall, Elebute and Lotspeich, 1968).

1.3.4 Enzyme Changes

The enzymes of nucleic acid metabolism are still very much under investigation and little is known of their control as yet. Some have already been mentioned in the section on DNA synthesis. Royce (1967) noted an increase in alkaline RNase (pH 7.8) in the renoprival rat kidney, but Malt (1969) in the mouse and Halliburton (1966) in the rat do not confirm this observation.

Lotspeich (1967) reports an increase in glutaminase in the renoprival rat kidney, though the increase was less than that seen in ammonium chloride acidosis. E-lysine acylase increases 35% in the first 3 days after unilateral nephrectomy (Paik and Kim, 1966).

Nowinski and Pigon (1967) found that isolated rat glomeruli contained all the enzymes of the Krebs cycle and these showed no change in enzyme activity or in ATP content during compensatory growth.

Nowinski et al. (1968) also report an increase of 100% in glutamic dehydrogenase activity in proximal and distal tubules 2 weeks after unilateral nephrectomy and an increase of 70% in alkaline phosphatase in the same time interval. Nowinski (1966) previously reported no increase in the enzymes of the Krebs cycle in the glomeruli of the kidneys undergoing compensatory hypertrophy. Johnson and Amendola (1969) have shown that the number of mitochondria increases in parallel with kidney growth; hence an increase in oxidation-reduction enzymes contained in the mitochondria might be expected. Succinic dehydrogenase is increased slightly 2 days after uninephrectomy but the activity of cytochrome oxidase and NAD cytochrome reductase is unchanged (Dies and Lotspeich, 1967; Rosenthal et al., 1962; Mascitelli-Coriandoli and Ierano, 1968). Glucose-6-Phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, the first enzymes of the Hexose monophosphate shunt, which yields ribose and NADPH, show marked elevations in compensatory growth of the kidney; the increase is 25% at 36 hours, falls off at 48 hours and rises again to 40% at 72 hours, then falls off again (Farquhar et al., 1968).

The enzyme Na-K-ATPase which is now thought to be responsible for operation of the sodium pump has been investigated and found to be elevated in the renoprival rat kidney. Katz and Epstein (1967a) found that the activity of this enzyme per milligram of kidney tissue rose 55% in the first 3 weeks after unilateral

nephrectomy, while sodium reabsorption increased 21%. Fanestil (1968) drained the ureter of one kidney into the peritoneal cavity and found that this did not increase the activity of Na-K-ATPase in the other, whereas unilateral nephrectomy and ureteral ligation did. This is said to prove that the cause of the increase is loss of functioning renal mass and not the increase in excreted load. However, it is possible that ureteroperitoneostomy itself may have had a depressant effect on Na-K-ATPase concentrations which would otherwise have been high.

1.4 THE CONTROL OF COMPENSATORY RENAL HYPERTROPHY

The mechanism controlling compensatory growth of the kidney is unknown. There are two principal theories :

(a) The Work Hypertrophy Theory

(b) The Humoral Theory.

1.4.1 The Work Hypertrophy Theory

This states that the stimulus to kidney growth in the renoprival state is the need to carry out duties normally shared between two, and that some early change in renal work might trigger renal growth. The study of kidney function following unilateral nephrectomy is obviously of some importance to this theory. There is scanty evidence of impaired renal function after unilateral nephrectomy, and since intermittent function of nephrons is unlikely (Pitts, 1968) the smaller number of remaining nephrons must work harder.

Renal Function after Uninephrectomy

Hartman and Bonfilio (1959) report that after uninephrectomy the mouse kidney becomes visibly engorged and within one minute rat glomeruli are hyperaemic. The blood flow to the solitary dog kidney increases rapidly, rises 50 to 100% and remains elevated for several weeks (Rous and Wakim, 1967). Glomerular filtration rate would be expected to increase immediately and it is easy to see how an increase in the rate of flow of filtrate could be a stimulus to renal growth. However, reported results are at variance. One difficulty of measuring G.F.R. is that there is a 10% error (Pitts, 1968). Therefore, early small changes obviously are difficult to interpret. Katz and Epstein (1967b) have shown that in rats over 250 gm. in weight there is no change in G.F.R. or tubular reabsorption of sodium until 3 days after unilateral nephrectomy. Of the original G.F.R. of 2 kidneys, 65% is restored at 3 days, 80% at one week and 88% at 2 - 3 weeks, and at this time G.F.R. and tubular reabsorption of sodium per gm. of kidney tissue are 27% greater than normal. During the first few days there is an increase in sodium excretion and urine flow, the possible reasons for which will be discussed in a later section.

Peters (1963), using rats of less than 250 gm., reports a decreased reabsorption of sodium and water and increased excretion of potassium within one hour of unilateral nephrectomy. He also found that the G.F.R. increased within 2 hours; by 12 hours the G.F.R. showed a 20% increase and Effective Renal Plasma Flow

(ERPF) showed a 12% increase. In dogs the GFR and ERPF shows a 100% increase in 24 hours and remains elevated by 50% for some weeks (Rous and Wakim, 1967; Bugge-Asperheim and Kiil, 1968a and 1968b).

It has been suggested that the early changes in renal function, in regard to GFR, sodium and water excretion, are the result of extrarenal regulatory mechanisms and that the later changes are due to growth of the kidney (Malt, 1969). This will be discussed more fully later.

Renal Function in Kidney Donors and Recipients

Some aspects of renal function are more easily observed in human beings than in animals. Kidney donors regain 70% of their original total GFR and 65 - 80% of their original total ERPF; the greater part of the increase occurs within the first week of operation. 80% of the original ERPF is restored in young donors in their twenties, but only 45 - 70% of those over 50 years of age. The blood urea nitrogen level rises from 13 to 16 mgm. per 100 ml. in the early weeks (Krohn, Ogden and Holmes, 1966; Donadio, 1967; Flanigan et al., 1968; Ogden, 1967). GFR, ERPF and the ability to maintain sodium balance in the transplanted kidney have been shown to be nearly identical to that of the donor's kidney (Blaufox, 1969). Thus, functional compensation occurs in both the recipient and the donors of transplanted kidneys and is obviously independent of nerve supply in the early stages, though the nerves do eventually regenerate (Norvell et al., 1969).

The time of onset of compensation is not settled, but a return of 74% of GFR and 69% of ERPF has been reported within one day, though this observation was made on a single patient (Sugino et al., 1967). Also, creatinine clearance was used to measure GFR and creatinine is not merely passively filtered but probably also actively excreted by the tubular cells; thus endogenous creatinine clearance is not a true reflection of GFR in man (de Wardener, 1967).

Hayslett et al. (1968) have performed elegant micropuncture studies in the rat which show how the compensations in sodium and water reabsorption occur. Urine flow increases 60% at 2 weeks after unilateral nephrectomy. Since the GFR almost doubles, sodium reabsorption must increase to maintain homeostasis. The transit time of the ultrafiltrate in the proximal tubule is unchanged as compared to controls; this means that reabsorption in this segment does increase for the flow down the tubule is proportional to the 96% increase in volume at this level at this time after uninephrectomy. In the distal tubule, however, transit time is increased 80%, presumably due to lengthening of Henle's loop. The distal tubule is doing more absorptive work per unit length. Gottschalk et al. (1969) report that fluid inulin/plasma inulin ratio is constant at all levels and glomerulotubular balance is maintained, and the rates of delivery and reabsorption of sodium at any point in the tubules change in proportion to the GFR. Malt (1969) reports that sodium excretion increases progressively as renal tissue is lost, and that the site of the control of sodium excretion is likely to be the distal

tubule, although Bricker (1967) suggests that the site is the proximal tubule. The possible mechanisms will be discussed in another section.

The kidney expends about 80% of its energy in reabsorbing sodium (Bricker, 1967), hence if the work hypertrophy theory is considered, it is reasonable to suppose that most of the increased work will be required to reabsorb sodium, and it is tempting to relate the reports on altered proximal tubular sodium reabsorption and altered RNA metabolism. However, there is no evidence that the alteration in RNA metabolism leads to new transport enzymes or other cytoplasmic proteins required for sodium transport. Furthermore, no change in tubular reabsorption of sodium occurs for 3 days after unilateral nephrectomy whereas growth is well established by that stage (Katz and Epstein, 1967b).

There is, however, some evidence for the Work Hypertrophy Theory, and several workers have investigated the effects of diverting urine to a site from which it would be absorbed and re-excreted. Hartman (1933), in the dog, found that an anastamosis between urinary bladder and distal end of the ileum caused enlargement of the kidneys in the next 6 - 8 weeks. Fortner and Kiefer (1948) transplanted dogs' ureters to the duodenum and found in some cases hypertrophy of the contralateral kidney. Bollman and Mann (1935) found that growth of the renoprival kidney was increased following transplantation of its ureter into the duodenum. However, Block, Wakim and Mann (1953) found that transplantation into the

duodenum did not cause increase in growth of the contralateral kidney. There is apparently some clinical evidence to support the claim that ureteric transplantation leads to renal hypertrophy. In the treatment of severe disease of the bladder it may be necessary to transplant the ureters. Uretero-sigmoidoscopy, i.e. transplantation into the colon, leads to severe complications and marked enlargement of the kidney, but the cause of the renal enlargement is far from clear. The urine is reabsorbed from the colon; the reabsorption of urea causes a rise in the blood urea. It is claimed that the reabsorption of chloride ions is important because they are absorbed at a faster rate than sodium ions and this, with the absorption of ammonium ions formed in the colon by the action of bacteria on urea, causes a hyperchloraemic acidosis, i.e. that ureteral transplantation is equivalent to a continuous infusion of ammonium chloride. Also, there is an intractable pyelonephritis (de Wardener, 1967). Now, there is no doubt that there is enlargement of the kidneys, but whether the enlargement and the hypertrophy are a response to the need to excrete more water, urea, sodium, chloride, hydrogen or ammonium ions or a compensation for tissue damage produced by pyelonephritis is not clear. Acidotic coma occurring as a complication is usually associated with an exacerbation of renal infection (de Wardener, 1967). This fact must throw in doubt the theory that the acidosis is due to accelerated chloride reabsorption, although the acidosis alleviates in a few months and this is said to be due to sodium and chloride being absorbed at the same rate.

Transplantation of the ureters to an isolated segment of ileum is not accompanied by such marked enlargement of the kidneys; but infection is less of a problem and the urine is in contact with the mucosa for a shorter time. Thus the clinical picture in this case does not explain the discordant results above.

Some workers have allowed the ureter to drain into the peritoneal cavity. Mason and Ewald (1965) found enlargement of the contralateral kidney. Goss and Rankin (1960) report no increase in the mitotic index after ureteroperitoneostomy. Simpson (1961) used ^{32}P to measure DNA synthesis and found no increase on the unoperated side. In the previous paragraph the question which could be raised against positive results is that they may be due to infection. In the case of ureteroperitoneostomy, Royce (1963) wondered whether irritation of the peritoneal cavity was the cause of negative results he obtained. He had noted inflammation of the peritoneum in all of his test animals. He therefore introduced a talc suspension into the peritoneum of animals which he unilaterally nephrectomised; this completely inhibited compensatory growth in the remaining kidney. It is possible, therefore, that the failure of some of the above experiments was due to associated inflammation.

The effect of ureteral ligation has also been investigated. The obstructed kidney shows hydronephrosis and an increase in weight and mitotic index. The opposite kidney shows a slight increase in weight and mitotic index (Hinman, 1943; Goss and Rankin, 1960; Mason and Ewald, 1965).

The importance of the results of the above is difficult to assess because all the experiments are open to the criticism that the growth obtained occurs in the presence of what may be a pathological state.

The other main body of evidence advanced in favour of the work hypertrophy theory is the response of the kidney to high protein diets. Reader and Drummond (1925) found kidney hypertrophy after some months in rats fed diets containing 45 to 90% caseinogen. Osborne et al. (1927) found that high protein diets produced kidney hypertrophy in as little as 8 days. McKay, McKay and Addis (1927) and McKay and McKay (1931) found that the extent of the hypertrophy varied directly with the protein content of the diet. More recently, similar results were obtained by Konishi and Brauer (1962). The liver responds similarly to high protein diets but not the spleen, adrenals, testis, thyroid, pituitary or seminal vesicles (McKay et al., 1928; Campbell and Kosterlitz, 1950; Leatham, 1945).

The response to unilateral nephrectomy is greater in animals fed a high protein diet (Moise and Smith, 1927; McKay, Addis and McKay, 1938; Reid, 1944; Konishi and Brauer, 1962). Konishi (1962) showed that a high protein diet increases the mitotic response to unilateral nephrectomy. Conversely, Mandel et al. (1950b) have shown that a protein-free diet will decrease the response to unilateral nephrectomy. The kidney weight, RNA and protein contents are decreased, although DNA content is not affected. As might be expected from the response to a protein-free diet,

starvation has a marked effect on the response to unilateral nephrectomy. Kurnick (1955) found that in normal, unoperated rats, starvation depresses total RNA and protein content after 2 - 3 days, but does not affect DNA content. Thus the cells are diminished in size but not in number. Sacerdotti (1896) found that starvation inhibited the response to unilateral nephrectomy. Hall and Hall (1952) confirmed this early finding. Williams (1962) found that the mitotic activity in the renoprival state was depressed in fasted animals. Royce (1963) and Reiter (1965) report that, after unilateral nephrectomy, the surviving kidney does not increase in weight or in DNA content if the animals are deprived of food and water. These workers also found that food and water deprivation caused normal kidneys to decrease in size and DNA content and conclude that, when animals are unilaterally nephrectomised and deprived of food and water, the two effects cancel out and the kidneys remain the same size as before. Halliburton and Thomson (1967) and Halliburton (1966) have investigated the effects of high protein diets and starvation on the response to unilateral nephrectomy in rats. The rise in RNA/DNA occurs whether or not the animal is starved, but the rise in protein/DNA content is abolished in starved animals. Malt and LeMaitre (1968) showed that in starved mice the increase in RNA/DNA was the same as in fed animals but that the peak was delayed from the second to the fourth day. Halliburton (1966) found that high protein diets produced renal hypertrophy. He found that a diet containing 45% casein produced a 15% increase in

renal mass and a 23% increase in RNA/DNA over control animals. He also found that the effects of a high protein diet were SIMILAR to those of unilateral nephrectomy. Halliburton also found that a diet containing 15% casein plus 40% gelatin produced a 60% increase in renal mass and a 30% increase in RNA/DNA, i.e. gelatin has a greater effect than casein on renal weight and composition. This phenomenon had been noted previously (Wilson, 1933). Reid (1947) found that whole proteins caused renal enlargement whereas mixtures of amino acids sufficient to double the nitrogen intake did not. Moore (1967) fed animals amino acids in the proportions and quantities found in the gelatin diet described above and found only 50% of the response. This he found was almost wholly attributed to glycine which, when making up 8% of the diet, produced a 25% increase in renal mass and increased both RNA/DNA and protein/DNA ratios.

It was originally claimed that the effects of high protein diets were due to increase in renal work required to excrete the additional urea. It is now known that urea is simply passively filtered by the kidney (Pitts, 1968) and has no effect on renal workload. Osborne et al. (1925) found kidney enlargement comparable to high protein diets by feeding large quantities of urea. Further, the fact that whole proteins are more effective than amino acid mixtures throws further doubt on the hypothesis. Gelatin is known to increase the GFR and was formerly used as a diuretic (Vogl, 1953) though it is not known whether this has anything to do with its effect in

causing renal growth. It is also possible that the effect is not due principally either to the quantity or the nature of the protein per se but to some contaminant.

Another possibility is that the response to a high protein diet is due to the extra acid which needs to be excreted. Sulphuric acid would be produced by metabolism of cysteine and methionine residues. Chronic metabolic acidosis produced by the addition of ammonium chloride to the diet or drinking water has produced a true growth of the kidney comparable to that of unilateral nephrectomy (Lotspeich, 1967; Halliburton, 1966), although the RNA/protein ratio is not altered. Further, the effect was found by Lotspeich to be additive to that of unilateral nephrectomy. Bignall et al. (1968) report that in compensatory renal hypertrophy the surviving kidney incorporates L-leucine at a faster rate than L-glutamine, whereas in NH_4Cl acidosis the opposite is true. This suggests that the mechanisms are not identical. Neither sodium bicarbonate (Lotspeich, 1965) nor ammonium citrate (Lotspeich, 1965; Halliburton, 1966) have any effect on kidney weight.

The effect of acidosis is interesting and appears to support the work hypertrophy theory, but one has to remember that the main function of the kidney is to reabsorb sodium. Numerous workers have investigated the effects of increasing the sodium chloride content of the diet. Osborne et al. (1927) found no growth of the kidney with a variety of salts in concentrations of up to 25%. Allen and Mann (1935) fed sodium chloride in a concentration of

15% by weight of the diet to unilaterally nephrectomized animals with no effect on kidney weight. Halliburton (1966) reports no effect with a diet containing 33% by weight of NaCl. However, Goss and Rankin (1960) found that substituting 1% NaCl for drinking water produced kidneys with a higher level of cell division than control animals at 5 days. They then nephrectomized the animals and found that the level of cell division in those given the salt in their drinking water was lower than in those given normal drinking water. They explain this by saying that in those given salt to drink hypertrophy has already occurred and the response to unilateral nephrectomy is correspondingly reduced. The reason for this does not seem to be clear. It is however interesting that solid NaCl has no effect whereas salted drinking water does produce an effect.

Water has not been greatly investigated for its overloading capacity. Zakharov (1961) injected 1 ml. of normal saline into mice and 5 ml. into rats and noted a decrease in mitotic activity in the kidneys 1, 2 and 3 hours later.

Thus, experimental work to date on the work hypertrophy theory does little to provide an indication of the control of compensatory renal hypertrophy. The mechanism of growth induced by high protein diets remains obscure and none of the most obvious causes seem satisfactory. The results with ammonium chloride are encouraging but do not seem to provide the key to the main mechanism involved in compensatory growth. The effects of salted drinking water, especially the fact that the response is not

additive to that of unilateral nephrectomy suggests that compensatory renal hypertrophy occurs as a response to disturbed salt and water balance; however, the phenomenon requires further investigation; as yet the only index of growth used has been the mitotic index and a more thorough investigation of the effects of saline drinking water on protein and nucleic acid metabolism would seem to be indicated.

1.4.2 The Possible Role of Humoral Agents

For many years now there has been a search in serum and tissue extracts for factors which control growth in specific organs (Weiss, 1952; Teir and Lahtiharyii, 1961; Bullough, 1965). There is also considerable evidence to the contrary; Alston ~~and Thomson~~ (1966), in a series of cross-circulation experiments on rats, found no evidence for humoral mediation of liver regeneration.

Sacerdotti (1896) reported that serum from nephrectomised dogs increased kidney size when injected into normal dogs. Cameron and Kellaway (1927) injected kidney homogenates into unilaterally nephrectomised guinea pigs, with no effect on the extent of the hypertrophy. However, Breuhaus and McJunkin (1932) found that kidney homogenates injected into normal and nephrectomised animals caused an increase in mitosis in both groups. Semenova (1961) reported similar results in mouse kidney.

Saetren (1956) reported that injected kidney macerate inhibited the response to partial nephrectomy in the mouse and Steuart (1958) found that kidney and liver homogenates both inhibited the response

to removal of one kidney and half of the other one; the kidney homogenate was the more potent. Stitch (1960) has also reported inhibition of kidney growth with liver homogenate in unoperated baby rats. These experiments are complex to interpret because of possible effects on the animals' diet and general health. This was illustrated by Williams (1962b) who found that injection of kidney macerate spread over the peritoneum depressed the response to unilateral nephrectomy but also diminished the animal's food intake; starvation for the same time also depressed the response. Liver or spleen macerates did not inhibit the response but did not affect the animal's appetite. Roels (1965) found that injection of kidney homogenates diminished the response to unilateral nephrectomy at 2 days, but noted no difference in body weights between test and control animals. He therefore disagrees with Williams' opinion that the result is due to technical complications, and believes the results are due to a hormone produced by the renal cells and controlling mitotic activity in the nephron. Unilateral nephrectomy might produce a reduction in the concentration of this hormone and thereby cause renal growth. This idea was first advanced as a general cause of growth in damaged tissues by Bullough (1965).

Goss (1963a) performed a series of experiments with fresh, cooked, frozen and trypsin dissociated kidneys which he injected intra-peritoneally to nephrectomised rats. He found they reduced the mitotic index by 50% but so did homogenates of liver, testis, spleen and blood. These results provide no evidence of tissue-specific growth regulating agents.

Attempts to demonstrate growth regulating factors in serum from nephrectomised animals by passive transfer of such serum to recipient animals have also given contradictory results. Both Goss (1963a) and Williams (1962) found no difference in the response to unilateral nephrectomy between rats injected with serum from other nephrectomised rats and those injected with serum from sham operated animals. These workers gave their animals one injection each. However, Lowenstein and Stern (1963) gave their animals (rats) 2 injections per day of 0.5 ml. serum for 4 days and reported an increase in incorporation of ^{3}H thymidine into the kidneys but not the liver of normal animals receiving serum from nephrectomised animals. These results suggest that it is difficult to transfer sufficient of the supposed stimulating hormone ~~from~~ TO the recipient ~~from~~ FROM the donor.

This difficulty can in theory be overcome by using parabiotic animals. Steuart (1958) combined rats in threes to make up parabiotic "triplets" and bilaterally nephrectomised each of the outer 2 animals. He reported that this resulted in a burst of mitotic activity in the intact animal. More recently, Kurnick and Lindsay (1968) and Johnson and Vera Roman (1968) report failure to demonstrate a humoral agent in parabiotic pairs, although Kurnick and Lindsay believe that such a fact~~or~~ exists. These experiments are difficult to interpret because of the small number of animals used and the possibilities of incomplete mixing of the circulations.

A special case of parabiosis is that between mother and foetus. Goss (1963b) found no evidence of compensatory renal hypertrophy in foetal kidneys 2 days after unilateral nephrectomy of the mother, performed on the 19th day of gestation. This negative result may be due to the fact that the foetal kidneys were already growing maximally; alternatively the humoral agent may not cross the placenta.

There are 2 reports of stimulation of growth of kidney cells growing in tissue culture being stimulated by serum from nephrectomised animals. Ogawa and Nowinski (1958) found that nephrectomy serum added to foetal kidney cells growing in tissue culture gave mitotic activities twice as high as normal serum. The factor was tissue-specific but not species-specific as it also affected puppy kidney cultures. Lowenstein and Lozner (1966) also found evidence of such a factor. The measured isotope incorporation into DNA in foetal kidney cultures inoculated with serum from unilaterally nephrectomised rats and report an increase in isotope incorporation between these cultures and those inoculated with serum from sham operated animals.

Malt (1969) believes that a growth promoting factor exists in serum and that it may be released from the kidney. Bump and Malt (unpublished data) have diverted the renal outflow of one kidney directly to the liver and found no change in RNA/DNA in either kidney during 2 weeks of observation. He concludes that either the factor is completely and promptly inactivated by the liver or it is independent of hepatic inactivation.

It has been suggested that the lymphatic system has a role in the control of compensatory renal hypertrophy. Fox and Wakim (1968) suggested that a humoral agent may act indirectly on the kidney by preventing differentiation of lymphocytes in the regional lymph nodes and thus inhibiting the manufacture of proteins that limit growth by a feedback mechanism. This theory is disputed for several reasons; firstly, compensatory renal hypertrophy occurs during intensive immunosuppression (Malt, 1969); secondly, compensatory growth is normal in mice with neonatal thymectomy and immature lymphatic systems (Bump and Malt); thirdly, compensation begins too fast for all the postulated steps if the control is to be regulated via a lymphocytic intermediary.

Other more specific substances have been suggested as affecting renoprival growth. Royce (1967) suggests RNase; as previously stated, this enzyme has been reported as increased in kidneys undergoing compensatory growth and it can easily be seen how it might affect RNA metabolism and so unbalance control mechanisms. Royce (1968) has also identified an abnormal α-globulin in the serum of bilaterally nephrectomised rats one hour after operation, and this may play some part in controlling the process, but its presence has yet to be proved after uninephrectomy.

In conclusion, therefore, the evidence for a humoral control of compensatory renal hypertrophy is by no means conclusive, although there is sufficient evidence to warrant further investigation.

1.4.3 Endocrine Effects on Compensatory Renal Hypertrophy

The extent to which the endocrine glands affect compensatory renal hypertrophy has, with most of the other aspects of the investigation of this phenomenon, given rise to some controversy. However, the role of the pituitary has been fairly definitely established. Fogelman and Goldman (1966) showed that in hypophysectomised rats the response to contralateral nephrectomy is scarcely different from normal in the first 48 hours, but soon declines. The profound inhibition in compensatory growth over the long-term is due mainly to lack of Growth Hormone (GH) (Astarabadi, 1962a). Hypophysectomy reduces kidney size in two ways : it results in a loss of kidney weight relative to body weight; it also results in a cessation of growth of the animals (Rolf and White, 1953). Since the decline in body weight secondary to hypophysectomy, but not all the decrease in renal weight is prevented by growth hormone, there may be another pituitary hormone responsible for the maintenance of renal weight. The loss of renal weight after ~~nephrectomy~~ HYPOPHYSECTOMY cannot be prevented by feeding a high protein diet (Leatham, 1945) and even if a rat is force-fed sufficient nutrients to prevent weight loss, renal mass remains depressed (Levin, 1944). Following unilateral nephrectomy there is an increase in thymidine incorporation in the pituitary (Nakamura, 1964) and an increase in acidophil cell number (Findley and Davis, 1956). This suggests reorganised pituitary function following unilateral nephrectomy.

Other pituitary hormones affect the compensatory process. Antidiuretic Hormone (ADH) produces a slight increase in mitotic activity, but if given in conjunction with a saline infusion there is a 40- to 50-fold increase in mitoses (Reiter, 1969; Devenyi, 1964). Hay (1946) showed that a purified thyrotrophic substance from the anterior pituitary increased kidney size in normal and hypophysectomised rats. Thyroxine increases kidney weight (Selye et al., 1945), the mitotic index (Pisi and Cavalli, 1955) and the protein and RNA content (Mandel and Revel, 1958). Thyroidectomy restricts renal growth though thiourea in drinking water does not (Reiter, 1969). Following unilateral nephrectomy columnar cells in the thyroid enlarge and follicles are depleted of colloid (Nakamura, 1964).

Adrenocorticotrophic hormone (ACTH) on the other hand has no effect on kidney weight and histology (Simpson, Li and Evans, 1946), and has no effect on the changes induced by hypophysectomy (Astarabadi, 1962b and 1963; McCreight and Reiter, 1965). The effect of adrenalectomy on compensatory renal hypertrophy was in disagreement until Goss (1965) showed that previously contradictory results (Goss and Rankin, 1960; Williams, 1962; Astarabadi, 1963) were due to variations in salt intake. Goss (1965) showed that adrenalectomy diminished the response if the animals were given water to drink, but had no effect if they were given saline drinking water. This has since been confirmed (Reiter and McCreight, 1965). It appears that an adequate sodium level is essential for

compensatory renal hypertrophy to occur and that the importance of the adrenals to the process is that the mineralocorticoids which they produce promotes sodium retention. The injection of deoxycorticosterone acetate has been shown to increase kidney weight and mitotic index (Ludden et al., 1941; Goss, 1965), and enhance thymidine incorporation in intact rats (Moraski, 1966). Castles and Williamson (1965) report that aldosterone increases RNA synthesis.

Several other steroid hormones exert an effect on compensatory renal hypertrophy. Cortisone acetate has been shown to inhibit completely the process (Goss and Rankin, 1960). The sex hormones exert an effect; if unilateral nephrectomy is performed before puberty in mice, the response shows more hyperplasia than if the operation is performed after puberty (Malt, 1969). In male mice, castration reduces renal weight (Fadial et al., 1967; Kassenaar et al., 1962), but oophorectomy has no effect on female rat kidneys (Malt, 1969). Nortestosterone abolishes the effect of castration on compensatory renal hypertrophy (Kassenaar et al., 1962; Fadial et al., 1967), and in intact animals injection of nortestosterone increases the kidney size and the rate of amino acid incorporation. (Kassenaar et al., 1962). Castrated mice do show some degree of compensatory growth, but RNA increases as kidney weight increases rather than at a faster rate as in normal mice or castrates receiving nortestosterone (Jelinek ET AL. 1964). Schaffenburg and McCullough (1953) report that large doses of oestrogens given to male mice have a significant depressant effect on compensatory growth following

unilateral nephrectomy and Shimkin et al. (1963) report a reduction in renal weight relative to body weight in male mice receiving a single injection of oestrogen.

There is no doubt, therefore, that many of the hormones secreted by the endocrine glands play some part in the control of compensatory renal hypertrophy. All the hormones discussed in the preceding paragraphs, except ADH, exert an influence on many aspects of an animal's metabolism, affecting most tissues and organs and, while the presence or absence of some of them has been shown to affect the extent of compensatory process, none is absolutely essential for its occurrence.

1.4.4 Extraordinary Influences on Kidney Growth

From time to time during the many years that compensatory growth of the kidney has been studied, various phenomena which are associated with growth of the kidney have come to light. Some of these may be related to the events occurring following removal of one kidney from an animal.

Argyris and Trimble (1964) have studied the effects of mild degrees of tissue damage. They report that minor degrees of damage to mouse kidney produces both ipselateral and contralateral increase in mitotic index. In the male mouse, sham operation produces a 5-fold increase in mitoses 2 days later and cortical damage to one kidney produced by needle puncture results in an 11-fold increase in mitotic index in the contralateral kidney.

Benitez and Shaka (1964) report that unilateral ureteral ligation evokes no mitotic response in the opposite kidney in the first 3 days, although there is an increase in mitoses in the obstructed kidney; Paulson and Fraley (1969) report a decrease in polyribosomes in acutely obstructed mouse kidney.

Other workers have sought a relationship between kidney growth and the regulation of blood pressure. Masson and Hirano (1969) found that unilateral renal artery occlusion produced contralateral hypertrophy only if the ligated kidney became atrophic. However, Lytton et al. (1968) report that ipselateral ischaemia at the time of contralateral nephrectomy produced a kidney which was 25% heavier than nephrectomy alone produced.

Reiter (1968) has investigated the effects of cold exposure on the renal mass in hamsters. He found that hamsters kept at temperatures between $4^{o}C$ and $7^{o}C$ showed marked hypertrophy of the kidney, and that the effect was additive to that of unilateral nephrectomy, in that there was a small effect due to cold exposure. Of course, the hamster is a hibernator and water-conserver (Reiter, 1969). Cold exposure also activates the thyroid and Johnson (1969) has shown that at this temperature in rats there is a marked increase in the BMR, and feels there may be also an increase in cardiac output and GFR. It may be that one of these associated phenomena is the cause of the renal hypertrophy which occurs.

Nervous and physical stimuli were investigated by Allen and Mann (1935). In the rabbit they noted that renal decapsulation and contralateral nephrectomy results in 40% increases in response at 6 months. Halliburton (1966) studied the effects of renal decapsulation at a shorter interval (48 hours) in the rat. He found no difference in the compensatory response in animals which occurred after unilateral nephrectomy as a result of decapsulation of the remaining kidney. Clearly, therefore, the presence of a capsule on the kidney has no effect on the extent of the compensatory response in the short time interval. These investigators also studied the effect of renal denervation on the compensatory response in the rabbit and again reported an increase. However, the presence of compensatory growth in denervated transplanted kidneys has virtually disposed of the renal nerve supply as an important stimulus to compensatory renal hypertrophy.

Threlfall et al. (1967) and Threlfall (1968) in the rat and Baserga et al. (1968) in the mouse report on the effects of folic acid injection on growth of the kidney. Injection of 5-50 mgms. of folic acid produces the greatest known growth response in the kidney. Malt (1969) reports that an injection of Vitamin B_{12} has the same effect. In the rat kidney RNA content increases by 50% in 24 hours and doubles by 4 days. DNA rises to a maximum of 70% greater than normal in 3-4 days; this rise in DNA is preceded by a doubling of RNA polymerase. After 36 hours the labelling of DNA and the number of cortical mitoses increases 16 times. In contrast with

unilateral nephrectomy only, the wet weight increases during the first day; thereafter both wet and dry weights increase by 60% and 30% respectively; also in contrast with events occurring after unilateral nephrectomy there is a marked increase in medullary mitosis. In the mouse DNA synthesis is increased by 20 hours, reaching a peak at 30-60 hours, by which time there is a 100-fold increase in mitoses. The effect is additive to that of unilateral nephrectomy in the rat (Threlfall et al., 1967).

The explanation for these changes in nucleic acid synthesis is not clear. If it were ever shown that the effect was pteridine-induced, this would be of immense value in the study of the control of nucleic acid synthesis. However, there is no evidence for this. On the other hand, most workers believe that the effect is purely a mechanical one. The early increase in wet weight and the increase in medullary mitosis, coupled with the production of similar effects by the intratubular precipitation of uranyl nitrate crystals (Taylor et al., 1968) and the finding that after injection of folic acid precipitates occur in the tubules (Bazerga et al., 1968; Taylor et al., 1968) would suggest that the experimental model is that of an acutely obstructed kidney.

1.5 THE SITUATION IN 1967

Much of the work reviewed in the preceeding paragraphs has been published between 1967 and 1969, and most of it, notably that of Malt and his co-workers in Boston, is of great value in clarifying the earliest changes in the kidney remaining following contralateral nephrectomy. However, in spite of this, the control mechanisms remain obscure and it seems necessary to look at various theoretical possibilities and some of the changes which might possibly occur following unilateral nephrectomy in an attempt to arrive at a reasonable hypothesis to explain the phenomenon of renoprival compensation.

SECTION 2

MATERIALS AND METHODS

2.1 GENERAL

2.1.1 Animals

Adult albino male rats from the departmental colony were used in almost all experiments. Their body weights were between 120 and 260 g. During experiments they were caged individually at 26°C and were fed ad libitum on stock diet (Table 2), except during dietary experiments when they were fed measured quantities of diet at regular times. They were allowed free access to water at all times.

In a few experiments, rabbits were used. These were adult Dutch strain animals, with body weights ranging from 1.6 kg. to 2.5 kg. These were housed individually and fed ad libitum on stock diet with free access to water at all times.

2.1.2 Isotopes

Isotopes were obtained from the Radiochemical Centre, Amersham. Tritiated L-leucine labelled at position 4 and 5 (Code No. TR K 170), ^{14}C Adenine, labelled (Code No. CFA 219) were used in most experiments. Both isotopes were stored at -10°C until required.

2.1.3 Diets

Halliburton (1966) reports that 15 g. of stock diet DAILY is sufficient food intake for rats of 150-200 g. body weight. The composition of stock diet (Diet 41, Bruce and Parkes, 1949) is given in Table 2. In dietary experiments, synthetic diets were used; the composition

of these is given in Tables 3 - 9. The calorie intake was controlled to provide 1,450 calories per square metre of body surface per day in order to maintain nitrogen balance (Munro and Naismith, 1953). The body surface was computed from the formula $S = 12.54 \times W^{0.60}$ sq. cm. where S is the body surface area and W the body weight (Lee, 1929). Consequently the animals were offered 15 g. of food per day, 7 g. at 10 a.m., initially after a 17-hour fast, and 8 g. at 4 p.m. All animal weights recorded were taken at 10 a.m.

2.1.4 Surgical Procedures

2.1.4.1 Unilateral Nephrectomy

Anaesthesia : Ether. Animal : Rat

Right unilateral nephrectomy was performed via a lumbar incision. After shaving, the skin and peritoneum were opened and the kidney manipulated through the incision, decapsulated and the pedicle ligated with cotton thread 5 mm. from the kidney. The peritoneum was closed with a continuous cotton suture and the skin with interrupted sutures. The kidney was washed in water and blotted on filter paper, weighed on a torsion balance and stored in the frozen state at $-70^{\circ}C$. In sham operations, the kidney was manipulated through the incision, handled for a length of time equivalent to that required for excision, returned to the peritoneal cavity and the wound closed as before.

2.1.4.2 Unilateral Nephrectomy and Partial Hepatectomy

Anaesthesia : Ether Animal : Rat

After shaving the skin, a midline abdominal incision was made just below the xyphoid process and the entire median lobe of the liver delivered through it. A loop of cotton thread was passed over the entire lobe and tightened. The lobe was then excised. The abdominal wound was closed in layers. Unilateral nephrectomy was then performed through a lumbar incision as previously described (2.1.4.1).

2.1.4.3 Unilateral Nephrectomy and Contralateral Renal Denervation

Anaesthesia : Induction - intravenous pentobarbitone (Nembutal) BP 0.75 ml/Kgm injected into the ear vein.

Maintenance - ether

Sterility : Full sterile precautions (gown, mask, gloves, sterile instruments and sutures).

Animal : Rabbit

Via a mid-line abdominal incision the left kidney was located and stripped of fat. All visible nerve fibres were cut and the fascial sheath stripped from the renal artery from the hilum of the kidney to the origin of the artery from the aorta. The fat was stripped from the upper 2 cms. of the ureter and all visible nerve fibres divided. The aorta was then stripped of all nerve fibres surrounding it for 1 cm. on either side of the origin of the renal artery. Care was taken not to damage the adrenal glands during this procedure (Allen and Mann, 1935).

The right kidney was now located, stripped of fat and the capsule removed. The pedicle was ligated with silk suture 1 cm. from the hilum and the pedicle divided. Packs used to displace were now removed and the wound closed in layers with silk sutures.

2.1.4.4 The Measurement of Carotid Blood Pressure

Anaesthesia : Pentobarbitone (Nembutal) BP 0.5 ml. 1 in 5 dilution injected intraperitoneally.

Manometer : The manometer consisted of a length of fine polythene tubing taped to a vertical board to form a U, open at one end and partially filled with mercury; the length of tubing between the mercury and the end to be inserted into the carotid artery was filled with heparinized saline (prepared by mixing 0.5 mls. of heparin BP 1000 iu/ml. and 4.5 mls. normal saline).

Animal : Rat

The neck was shaved and an incision made along the right lateral aspect of the trachea and the right carotid artery was located and exposed over a length of 1.5 cms. Proximally a small clip was used to clamp the artery. Distally a loop of thread was placed under the artery and by gentle traction on this loop the artery was kept slightly stretched. Using watchmaker's forceps, the fascial sheath was stripped from the artery and then, using fine scissors, with the aid of a binocular magnifying lens, the artery was incised but not completely divided. The free end of the polythene tubing was

inserted into the artery and pushed proximally, then tied in place with a suture round the artery. The fine clamp was now released and blood pressure recorded.

B.P. = Alteration of height of mercury column x 2 mm/.Hg.

Unilateral nephrectomy was now performed as described previously and the blood pressure again recorded. Serial readings were made for 30 minutes and the animal sacrificed.

2.1.4.5 Withdrawal of Blood Samples

Venous: Anaesthesia : Ether Animal : Rat

The neck was shaved and opened over the jugular vein and samples withdrawn with a syringe and needle. The wound was closed with cotton sutures.

Animal : Rabbit

The ear vein was nicked with a scalpel, with anaesthesia, and the blood collected into a test tube.

Arterial: Anaesthesia : Ether Animal : Rat

The abdomen was opened with a left paramedian incision, the stomach and spleen displaced to the right and blood was withdrawn with a syringe and needle from the aorta just below its exit from the diaphragm. The animal was then sacrificed.

2.1.4.6 Injection of Urea Saline Mixture

Anaesthesia : Trilene Animal : Rat

The mixture was injected into the tail vein as described by Thomson (1970).

2.1.5 Tissue Dry Weight Analysis

This was estimated by weighing the tissue on a previously weighed watch glass, mincing it with scissors and re-weighing after 3 days in an incubator at $70^{o}C$.

2.1.6 Statistical Analyses

These were performed according to Snedecor (1949). Where P is used, it is in the conventional sense, e.g. $P < 0.01$ indicates a significance at the level of 1%.

TABLE 2

ANALYSIS OF DIET 41 (BRUCE AND PARKES, 1949)

Protein	13.7%
Fat	3.5%
Carbohydrate	49.0%
Fibre	1.5%

TABLE 3

COMPOSITION OF SEMI-SYNTHETIC DIETS

The physiological calorie VALUES of the substances used in the diets are :

Protein	4	calories per gram
Carbohydrate	4	calories per gram
Fat	9	calories per gram
V.M.R.*	3.5	calories per gram
Amino acids	4	calories per gram
Citrate	2.8	calories per gram
Inorganic salts	0	calories per gram

The diets used had a calorie equivalent of 4.18 calories per gram. Animals were offered 15 g. of food per day; where inorganic ions were added to the diet, the quantity offered was altered to give the equivalent of 15 g. of diet of 4.18 calories per gram.

* see Table 4

TABLE 4

COMPOSITION OF VITAMIN-MINERAL-ROUGHAGE (V.M.R.) MIXTURE
(Munro, 1949)

Sodium chloride	32.5 g
Salt Mixture "446" *	130.0 g
"Vitamins in Starch" **	250.0 g
Agar Powder	62.5 g
Margarine	77.5 g

1 g α-tocopherol acetate was mixed with 14 ml. radiostoleum (B.D.H.). 0.8 ml. of this was mixed with the above.

* see Table 5

** see Table 6

TABLE 5

COMPOSITION OF SALT MIXTURE "446"

Na Cl	243.2	g
Potassium citrate	533.0	g
KH_2PO_4	174.0	g
Ca H PO_4	800.0	g
Ca CO_3	368.0	g
Ferric citrate $3H_2O$	36.0	g
Cu SO_4 $5H_2O$	0.4	g
Co Cl_2 $6H_2O$	0.2	g
K_2 Al_2 $(SO_4)_4$ $24H_2O$	0.2	g
NaF	0.002	g
Mg CO_3	92.0	g
Mn SO_4	2.8	g
K I	0.1	g
Zn CO_3	0.1	g

TABLE 6

COMPOSITION OF VITAMINS IN STARCH

Pyridoxine hydrochloride		25	mg
Riboflavin		25	mg
Thiamine hydrochloride		25	mg
Nicotinic acid		100	mg
Menaphthone		5	mg
Biotin		5	mg
Calcium pantothenate		200	mg
Para-aminobenzoic acid		500	mg
Inositol		1.0	g
Choline chloride		10.0	g
Folic acid		Trace	
Potato starch	to	500	g

TABLE 7

COMPOSITION OF DIETS CONTAINING AMINO ACID SUPPLEMENTS

	Control	Glycine	Arginine HCl	Arginine
Casein (g)	120	120	120	120
Margarine (g)	42	42	42	42
Glucose (g)	429	398	310	330
Potato starch (g)	129	96	68	80
V.M.R. (g)	80	80	80	80
Amino acid (g)	0	64	180	148

The amino acid supplements were added to make them isomolecular with a diet containing 8% by weight of glycine.

TABLE 8

COMPOSITION OF DIETS
CONTAINING INORGANIC AND ORGANIC SALTS

	Control	Na Cl	K Cl	K Citrate	Na Citrate	Glycine + K Citrate
Casein	120	120	120	120	120	120
Margarine	42	42	42	42	42	42
Glucose	429	429	429	399	407	369
Starch	129	129	129	96	96	70
V.M.R.	80	80	80	80	80	80
Amino acid						64
Salt		9.5*	16.5^{+}	80^{++}	73^{++}	80^{++}

* This gives a total Na Cl content of 2% by weight

\+ This makes the osmolarity of K^+ in the diet equal to that of Na^+ in the Na Cl diet

++ These diets are iso-osmolar as far as the inorganic supplement is concerned and are iso-calorific.

TABLE 8a

COMPOSITION OF DIETS
CONTAINING SODIUM ACETATE AND POTASSIUM ACETATE

	Na Acetate	K. Acetate
Casein	120	120
Margarine	42	42
Glucose	385	385
Starch	112	112
V.M.R.	80	80
Salt	112*	80+

* This gives a total Na^+ content equal to the diet containing 2% by weight Na Cl.

\+ This gives a total K^+ content equal to that of the high K Cl diet shown in Table 8.

The diets shown above are iso-calorific.

TABLE 9

COMPOSITION OF HIGH AND LOW FAT DIETS

	Control	High Carbohydrate Protein-free	*High Fat	*High Fat Protein-free
Casein	120	-	87	-
Margarine	42	42	90	90
Glucose	429	509	171	496
Starch	129	169	94	149
V.M.R.	80	80	58	80

* These diets provide 33% of their calories as fat and 13 g. provides 63 calories, which are provided by 15 g. of the other diets. Therefore, rats were fed 13 g. of the high fat diets per day instead of the usual 15 g.

2.2 ANALYSIS OF BLOOD

2.2.1 Estimation of Plasma Sodium

The reagents were as follows :

A. Stock sodium solution : 58.5 g. Na Cl/litre

B. Standard sodium solutions :

75 ml. stock sodium solution was made up to 500 ml. with distilled water. For use, this was diluted 1 in 1000 with distilled water to give a concentration of 150 milliequivalents sodium ion per litre.

The plasma, diluted 1 in 1000 with distilled water was sprayed into the flame of an EEL flame photometer (Evans Electroselenium Ltd., Halstead, England), and the light output compared with a calibration curve previously prepared using standard sodium solution. Each point represented the mean of 10 readings.

2.2.2 Estimation of Serum Calcium

Principle:

Calcium is precipitated from serum by oxalic acid and, after washing, the calcium oxalate is dissolved in sulphuric acid and combined oxalic acid titrated with standard potassium permanganate.

Reagents:

A. Ammonium oxalate - half saturated solution

B. Dilute ammonia - 1 in 50 dilution of "880"

C. I N $H_2 SO_4$

D. 0.01 N $KMnO_4$

Method

2 ml. of serum was pipetted into a 15 ml. conical centrifuge tube and 4 ml. of half-saturated ammonium oxalate added. The contents of the tube were mixed thoroughly, allowed to stand for 30 minutes and then centrifuged at 1000 g for 10 minutes. The supernatant was discarded without disturbing the precipitate and the tube drained on filter paper; finally, the mouth of the tube was wiped to remove any fluid left. Now 3 ml. dilute ammonia was added from a pipette so that the precipitate was well stirred up and a further 2 ml. dilute ammonia added to wash down the sides of the tube. After centrifuging at 1000 g for 10 minutes, the supernatant was discarded and the tube drained on filter paper; this washing and draining was repeated once. The precipitate was dissolved in 2 ml. N H_2 SO_4 by heating in a water bath at 80°C and then titrated against 0.01 N K Mn O_4, keeping the tube at 80°C. The end-point was a permanent pink colour (2 minutes). A blank titration was carried out using 2 ml. N H_2 SO_4.

1 ml. 0.01 N K Mn O_4 = 0.2 mg. calcium

mg Ca^{++}/100 ml. serum = ml 0.01 N K Na O_4 x 10.

2.2.3 Haematocrit

Blood haematocrit was obtained using the Hawksley micro-haematocrit centrifuge (Hawksley and Son Ltd., Lancing, England). Blood was drawn into a capillary tube, the unfilled end sealed in a flame and the tubes centrifuged for 5 minutes; the percentage packed cell volume was obtained by using a micro-haematocrit reader.

2.2.4 Amino Acid Analysis

Blood was withdrawn and centrifuged at 1000 g for 10 minutes and the plasma amino acid pattern obtained on an Amino Acid Analyser.

2.2.5 Estimation of Acid-Base Balance

Acid-base balance was estimated using Micro-Astrup equipment (Radiometer A/S, 72 Emoripven, Copenhagen). Arterial blood was obtained as described in Section 2.14.5 and 3 capillary tubes were filled. The first sample was drawn by suction via the polyethylene micro-pipette into the glass electrode, the circuit completed by inserting the end of the pipette into a KCl bridge and the pH read. The second and third samples were equilibrated to 30 mms. Hg. CO_2 and 60 mms. Hg. CO_2 respectively in the containers provided for this purpose on the machine, and the pH values of these samples were taken as for the first sample.

From a Siggard-Anderson curve nomogram (Siggard-Anderson and Engel, 1960) the actual pCO_2, Buffer Base, Standard Bicarbonate and Base Excess of the sample was now calculated.

2.2.6 Estimation of Blood Ammonia

Principle

Ammonia determination was carried out using the enzymatic method described by Schmidt and Schwartz (1965), which has been found to compare favourably with the classical micro-diffusion technique of Conway (1957) (Mütting et al., 1968).

$$\alpha\text{-ketoglutarate} + NH_4^+ + NADH + H^+ \xrightarrow[GLDH]{} \text{glutamate} + NAD^+ + H_2O.$$

The enzyme Glutamate Dehydrogenase (1.4.1.2) catalyses the reaction, and the NH_4^+ content is directly proportional to the NAD^+ formed. The NAD^+ formed can be measured spectrophotometrically by the extinction decrease at wavelengths 340 mμ and 365 mμ respectively.

Reagents

1. TRIS BUFFER 0.2 M pH 7.6

   This was prepared from 0.8 M Tris Buffer (9.68 g Tris/100 ml) and 0.8 M H Cl. 50 ml Tris Buffer were mixed with 32.5 ml H Cl and made up to 200 ml with distilled water. This gives Tris Buffer of pH 7.6.

2. ∝- ketoglutarate 0.08 M

   This was prepared from ∝- ketoglutaric acid. 116.8 mgms were dissolved in water, 1.6 mls I N Na OH added to bring to pH 7 and made up to a total volume of 10 mls.

3. NADH 0.3 M

   i.e. 20 mgms/ml (in 1% $NaHCO_3$)

4. Glutamate Dehydrogenase-Ammonia free (EGAH 15324, Boehringer, Mannheim).

   GLDH-5 20 mgms./2ml.

5. $K_3PO_4 \times 3.H_2O$ 0.25 M

   i.e. 6.65 g/100 ml.

6. 10% TCA (Trichloracetic acid).

7. TEST MIXTURE

1. 29 ml Tris Buffer pH 7.6
2. 2 ml 0.08 M ∝-ketoglutarate
3. o.6 ml NADH in 1% Na HCO_3

Method

2 ml of whole blood (with 0.02 ml heparin) was withdrawn and placed immediately into 2 ml ice-cold 10% TCA without allowing the tip of the syringe to touch the TCA. This mixture was immediately centrifuged at 1000 g for 10 minutes. * Immediately after centrifuging, 1 ml of supernatant was transferred to a 10 mm cuvette and 1 ml $K_3\ PO_4$ added for neutralization. Then 1.5 ml of Test Mixture was added and the reaction started with 0.04 ml GLDH-5. At the end of the reaction, generally 30-40 minutes, the extinction difference of a blank (distilled water instead of blood) was deducted from the extinction difference of the test sample. The NH_4^+ content of the sample is calculated thus -

$$\frac{E_{365} \times 18 \times 3.54}{3.3 \times 2} = E \times 9.65 = \mu g\ NH_4/ml$$

$$\therefore\ NH_4^+/100\ ml = E \times 9.65 \times 100\ \mu g$$

* One step in the sequence must follow another immediately in order to keep secondary hydrolysis to a minimum.

2.3 CHEMICAL ESTIMATIONS

2.3.1 Extraction of RNA and DNA

The method was modified from that of Schmidt and Thannhauser (1945) (Munro and Fleck, 1960).

Reagents

A. 0.6 N perchloric acid (PCA)

B. 0.2 N perchloric acid (PCA)

C. 0.3 N potassium hydroxide (KOH)

The tissue was homogenised in 49 volumes ice-cold distilled water in a Nelco blender at 0°C for 2 minutes. A 5 ml aliquot of this homogenate (containing approximately 100 mgm tissue) was pipetted into a centrifuge tube and 2.5 ml 0.6 N PCA was added. After thorough mixing, the tube was allowed to stand at 0°C for 2 minutes, then centrifuged at 1000 g for 10 minutes. The precipitate of nucleic acid and protein was washed twice with 5 mls 0.2 N PCA. The supernatant and washings were discarded. The excess PCA was drained off, the sides of the tubes carefully dried and 4 ml 0.3 N KOH added. Digestion was then carried out in a shaking water bath at 37°C for 1 hour, at the end of which time the samples were chilled in ice and DNA and protein precipitated by the addition of 5 ml 0.6 N PCA. The precipitate was collected by centrifugation and washed twice with 5 ml 0.2 N PCA. The supernatant and washings were combined, made up to 50 ml and a final concentration of 0.1 N PCA. This was the RNA fraction. The precipitate was dissolved in 5 ml. 0.3 N KOH and made up to 25 ml and a final

concentration of 0.1 N KOH. This was the DNA fraction. A flow sheet summarizing the procedure is given in Fig. 1.

2.3.2 Estimation of RNA in the Fraction

The RNA content of the perchloric acid extract was estimated on the basis that an extinction of 1000 at a wavelength of 260 mμ, read with a light path of 1 cm., corresponds to a concentration of 3.412 μg. ribonucleic acid phosphorus (RNAP) per ml. (Fleck, 1965).

2.3.3 Estimation of DNA in the Extract

The DNA content of the extract was estimated by the method of Ceriotti (1952, 1955).

Reagents

A. Indole 0.4% (w/v) in distilled water

B. Concentrated hydrochloric acid (Analar SG 1.19)

C. Chloroform (Reagent Grade)

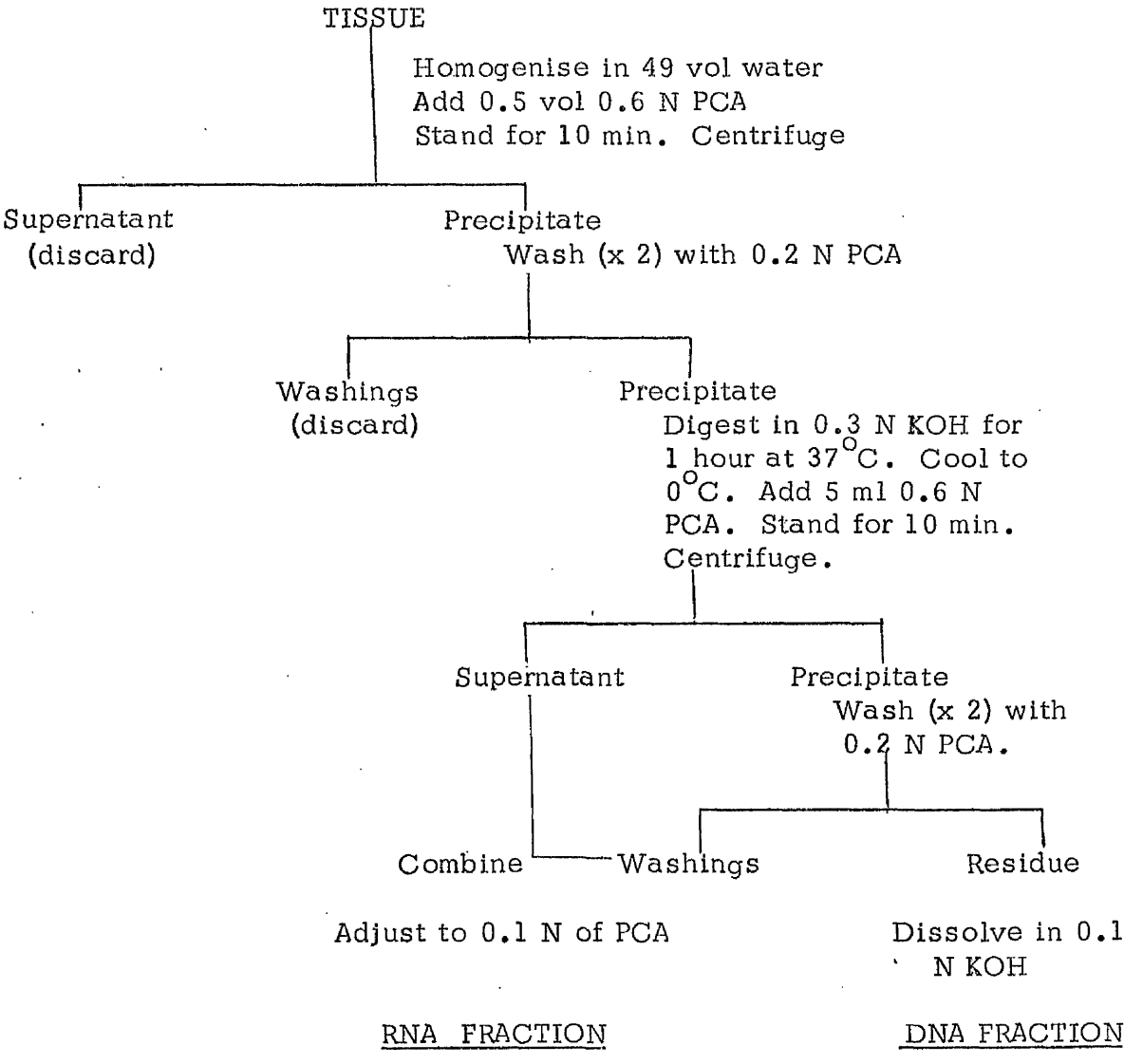
D. DNA standard

The DNA used was a purified sample of the sodium salt of calf thymus DNA prepared by the method of Kay, Simmons and Dounce (1952). About 20 mg (in fact 19.3 mg) DNA were dissolved in distilled water with a drop of N NaOH to help solution, the final volume being 50 ml. A 1 ml aliquot of this solution was diluted with 0.5 N PCA, heated to 70° for 20 minutes to redissolve any precipitated DNA, and made up to 50 ml.

FIG. 1

PROCEDURE FOR THE SEPARATION OF RNA AND DNA

All operations were performed at 0°C unless otherwise stated.



2 ml. of the DNA solution, 1 ml. indole, and 1 ml. concentrated HCl were thoroughly mixed in a 10 ml. ground glass stoppered test tube and placed in a boiling water bath for 10 minutes. After rapid cooling in ice, the solutions were extracted 3 times with 4 ml. portions of chloroform, shaking for about 45 seconds after each chloroform addition. After the third extraction, the tubes were centrifuged for 10 minutes at 500 g at room temperature to aid separation of the aqueous and chloroform layers. The extinction of the aqueous layer was read at 490 mμ on a Beckman DB spectrophotometer. Blanks consisting of 2 ml. distilled water and 2 ml. standard DNA solution were also carried through this procedure.

2.3.4 Estimation of Protein

Estimation of protein was carried out by the method of Lowry, Rosebrough, Farr and Randall (1951).

Reagents

A. 1% (w/v) sodium carbonate in 0.1 N NaOH

B. 0.5% (w/v) cupric sulphate ($CuSO_4\ 5H_2O$) in 1% sodium or potassium tartrate

C. Alkaline copper solution (1 ml. of reagent B in 50 ml. reagent A. This solution was made up fresh each day).

D. Folin-Ciocalteau phenol reagent (British Drug Houses Ltd., Poole, England).

This was diluted with distilled water to make it 1 N in respect to acid.

The tissue was homogenised in 49 volumes ice-cold distilled water in a Nelco blender. The homogenate was diluted 1 in 50 with distilled water to give a final dilution of 1 in 2,500. 5 ml. of reagent C were added to 1 ml. of the diluted homogenate. After 10 minutes, 0.5 ml. reagent D was added with vigorous shaking. After standing at room temperature for 30 minutes, the extinction of the solution was read at 750 mμ in a Unicam SP500 spectrophotometer. The assay was calibrated using a standard curve obtained with solutions of bovine serum albumen varying from 25 mg/ml to 250 mg/ml. The curve is linear up to 500 mg/ml.

2.3.5 Extraction of Lipid

The method used was based on that of Folch, Lees and Sloane-Stanley (1957).

Reagents

A. Chloroform-methanol mixture, 2 : 1 by volume

B. 0.73% (w/v) sodium chloride

C. Pure solvents "upper phase." This was the upper phase of a mixture of chloroform, methanol and 0.58% (w/v) sodium chloride in the proportions 8 : 4 : 3 by volume. The phases were separated by centrifugation.

A 1 in 5 homogenate of kidney in distilled water was prepared in a Nelco blender. 10 ml. of the chloroform-methanol mixture was then added to 0.5 ml. of the homogenate in a 30 ml. ground glass stoppered centrifuge tube. The tube was shaken thoroughly at intervals for 15 minutes. The non-aqueous phase was quantitatively

transferred to another 30 ml. ground glass stoppered centrifuge tube. The aqueous phase was then re-extracted with a further 10 ml. chloroform-methanol mixture. The 2 extracts were combined, shaken with 4 ml. 0.73% sodium chloride and centrifuged at 1000 g for 10 minutes. The upper layer was removed, and discarded. Carefully, without disturbing the interface, the walls and interface were washed with 4 ml. of pure solvents "upper phase". This procedure was repeated twice, the washings being discarded each time. The washed extract was quantitatively transferred to a 25 ml. measuring cylinder. The centrifuge tube was washed twice with approximately 3 ml. methanol. The washings were transferred to the measuring cylinder and made up to the mark with methanol.

2.3.6 Estimation of Lipid

The method used was that of Bloor (Paul, 1958).

Reagents

A. Chromic acid.

A 3 ml. aliquot of the methanol extract was evaporated to dryness at 70°C in a water bath. 3 ml. chromic acid was now added and the samples placed in a boiling water bath for 10 minutes. After cooling (slow), 3 ml. distilled water was added and the samples thoroughly mixed, and the extinction read on a SP600 spectrophotometer at 562 mμ. Blanks consisting of 3 ml. methanol and standard solutions of cholesterol from 0.25 mg/ml. to 1.5 mg/ml. were also carried through this procedure. The standard curve is linear.

2.4 ENZYME ANALYSIS

2.4.1 Assay of Amidotransferase (2.6.2.1)

Principle

Amidotransferase catalyses the reaction :

Arginine + Glycine $\rightleftharpoons$ Ornithine + Guanido acetic acid.

Glycine may be replaced by other substances and in the amidotransferase assay devised by Walker (1958) glycine is replaced by hydroxylamine :

Arginine + Hydroxylamine $\rightleftharpoons$ Ornithine + Hydroxyguanidine

Hydroxyguanidine may be estimated spectrophotometrically by allowing it to react with $Na_3Fe(CN)_5NH_3$.

Reagents

A. 1 M Phosphate Buffer pH 7.4

B. 1 M L-Arginine HCl

C. 2 M Hydroxylamine HCl, neutralised in 2 M KOH
The hydroxylamine HCl was dissolved in 2 M KOH and stored frozen until required.

D. 1 M L-Ornithine HCl

E. Acetone (Analar)

F. 1% $Na_3(Fe(CN)_5\ NH_3)$

G. 0.1 M Potassium Phosphate Buffer pH 7.4, containing 0.001 M Versene

H. 1 M Potassium Phosphate Buffer pH 7.0

J. 30% Trichlor acetic acid (TCA)

Method

A 15% homogenate of kidney in 0.1 M potassium phosphate buffer containing 0.001 M versene was prepared by mixing in a Tenbroek glass homogeniser, 10 strokes of the pestle being sufficient for thorough homogenisation. Centrifuge tubes were prepared containing 0.5 ml. homogenate, 0.1 ml. 1 M phosphate buffer pH 7.4, 0.1 ml. 1 M L-arginine HCl, 0.3 ml. 2 M hydroxylamine HCl (neutralized with KOH as described). A blank tube contained 10 mg. L-ornithine HCl in place of L-arginine HCl. All test and blank tubes were then incubated at 37°C for 90 minutes, at the end of which time the reaction was ended by the addition of 1.0 ml. distilled water + 0.4 ml. 30% TCA. The tubes were allowed to stand for 10 minutes, then centrifuged at 1000 g for 10 minutes. 1.5 ml. aliquots of the supernatant solutions were added to 13 x 100 mm. test-tubes, to which were added 0.5 ml. water, 2.0 ml. 1 M potassium phosphate, 0.3 ml. acetone, 0.3 ml. 1% $Na_3Fe(CN)_5NH_3$. The tubes were mixed and, after 10 minutes, the extinctions were read at 480 mμ on a Bausch and Lomb Spectronic 20 colorimeter.

The results were expressed in terms of O.D units per 100 mg. kidney tissue and as O.D units per kidney. In order to express the results in terms of ug. hydroxyguanidine formed, it is necessary to synthesise a stock standard of hydroxyguanidine; this is a laborious procedure and is unnecessary for the present investigation.

2.5 TECHNIQUES USED IN TISSUE CULTURE

When embryonic tissues are associated by treatment with tryptic enzymes, the individual cells are able to reaggregate to form the essential architecture of the tissue of origin (Mosconna, 1952 a and b; 1957). Mosconna has shown that the ability to react in this way decreases with increasing embryonic age. Dickson and Leslie (1963) devised a technique to allow adult guinea pig kidney cells to aggregate using micropore filters which formed the base of filter wells set in Howell organ culture units. They studied the metabolism and reaggregation over 6 to 10 days; organisation was well-developed at 4 days and by 10 days a tubule was easily distinguished.

A modification of the method of Dickson and Leslie was used in the present investigation, to maintain adult rat kidney cells in a viable but non-proliferating state.

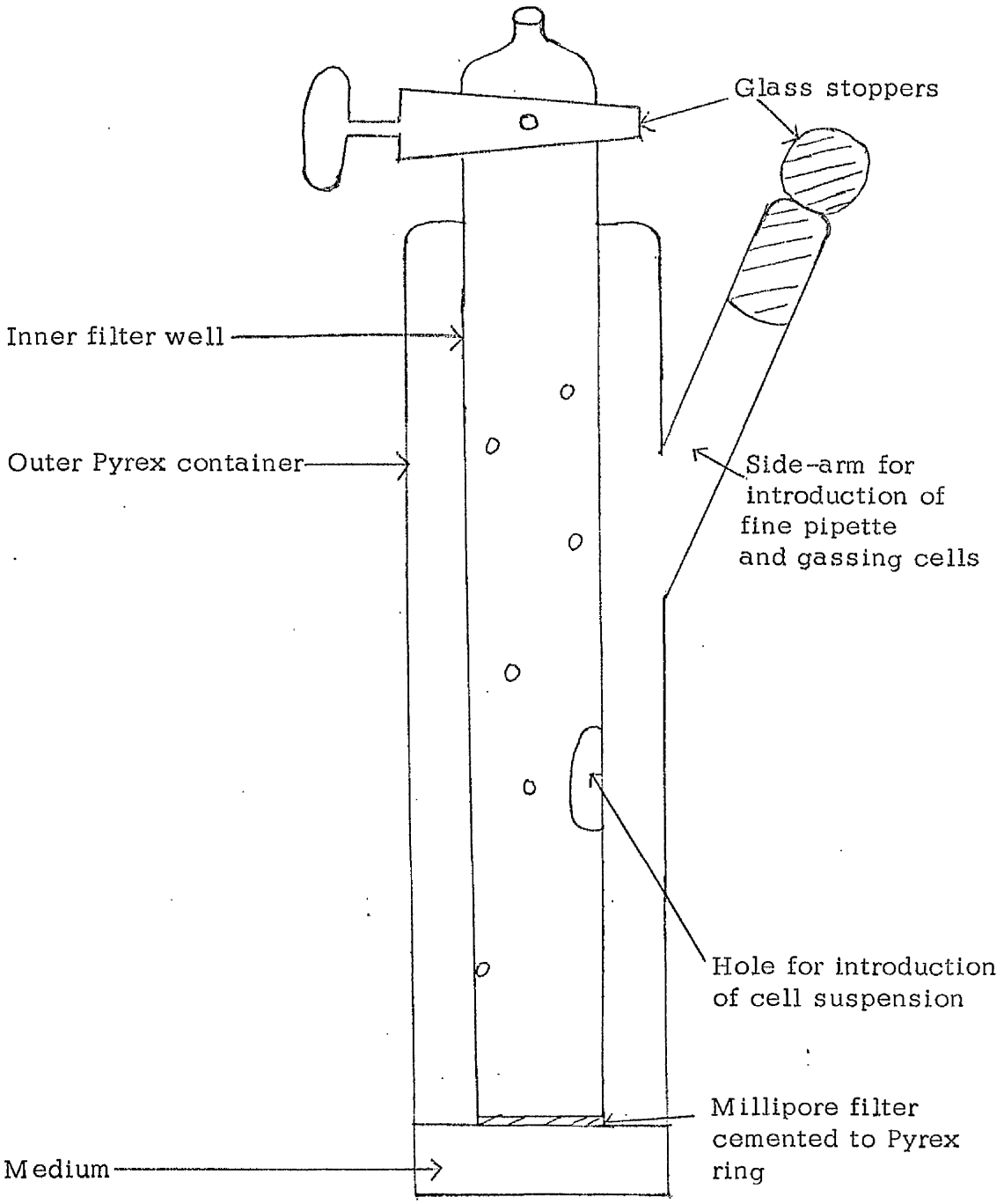
2.5.1 Materials

2.5.1.1 Filter Well Apparatus

Dickson and Leslie (1963) describe a technique for maintaining cell suspensions on millipore membranes cemented on to Pyrex rings to form filter wells. A modification of this technique (Dickson, 1967) was used in the present investigation. A diagram of the apparatus is shown in Fig. 2.

FIG. 2

FILTER WELL APPARATUS



Volume of vessel from base to level of millipore filter = 1.5 ml.

The filter wells were manufactured in such a way that the parts were interchangeable between different sets and so that a constant volume (1.5 ml.) of medium just touched the base of the millipore filter.

Preparation for Use

The filter well apparatus must be prepared in such a way as to minimise the risk of contaminants toxic to cells remaining. Before the first use of the apparatus it must be steeped overnight in chromic acid, rinsed in water, then washed in 0.1 N NaOH to neutralise traces of chromic acid remaining, rinsed again in water, then thoroughly washed in distilled water before being dried in a hot air oven at 70°C. Thereafter the apparatus should be handled using polythene gloves, to minimise contamination with sweat. The ring at the base of the well is moistened with Millipore No. 1 Cement and a filter applied, using forceps. The stop-cocks are greased with a fine coating of High Vacuum Grease and the parts autoclaved at 115°C and 15 lbs. per square inch for 20 minutes, after wrapping in greaseproof paper.

2.5.1.2 Millipore Filters (Millipore Ltd.)

Diameter - 13 mm. Pore size - 1.2 μ used in all experiments.

Millipore Cement - No. 1

2.5.1.3 Other Apparatus

A. 1 sterile 100 ml. beaker

B. 1 sterile 100 ml. conical flask with ground glass stopper

C. 1 sterile Teflon coated magnet

D. 1 magnetic stirrer

E. Sterile surgical instruments :

1. 1 pair of scissors + 1 pair of dissecting forceps for opening skin and peritoneal cavity.
2. 1 pair of fine scissors + 1 pair of tissue forceps for handling, removing and mincing kidney.

2.5.1.4 Calcium and Magnesium Free Saline (Saline A; Rinaldini, 1959)

A.	Phenol Red	0.4g
B.	Sodium Chloride	160 g
C.	Potassium Chloride	8.0g
D.	Glucose	20.0g
E.	Sodium Bicarbonate	7.0g

The above constituents were dissolved in double distilled water and made up to 1000 ml, and stored at -10°C. For use, the saline was diluted 1 in 20 with deionized distilled water, then sterilized by filtration.

2.5.1.5 Trypsin 1%

The disaggregating reagent consisted of trypsin 1% (Difco Laboratories Inc. : 1 : 250 trypsin) solution in saline A. 0.25 g. trypsin was dissolved in saline A with the aid of a few drops of 1 Molar HCl, the solution made up to 250 ml. with saline A and the pH readjusted with 1 N NaOH. The trypsin solution was then sterilized by filtration and stored in the frozen state until required.

2.5.1.6 Modified Eagles Medium (EHM)

The medium was prepared as a ten-fold concentrate, then diluted with sterile deionized distilled water just before use. The composition of the medium is given in Table 10.

2.5.1.7 Insulin - Boots Once Crystallized Insulin

Insulin .1 u/ml. was added to the medium before use.

2.5.1.8 Calf Serum (Flow Laboratories Ltd.)

5% calf serum was added to the medium before incubation of the cultures. In experiments with rat plasma this was added instead of, not as well as calf serum.

2.5.1.9 Rat Plasma

This was obtained as previously described and was added to the medium after 3 days' incubation with calf serum.

TABLE 10

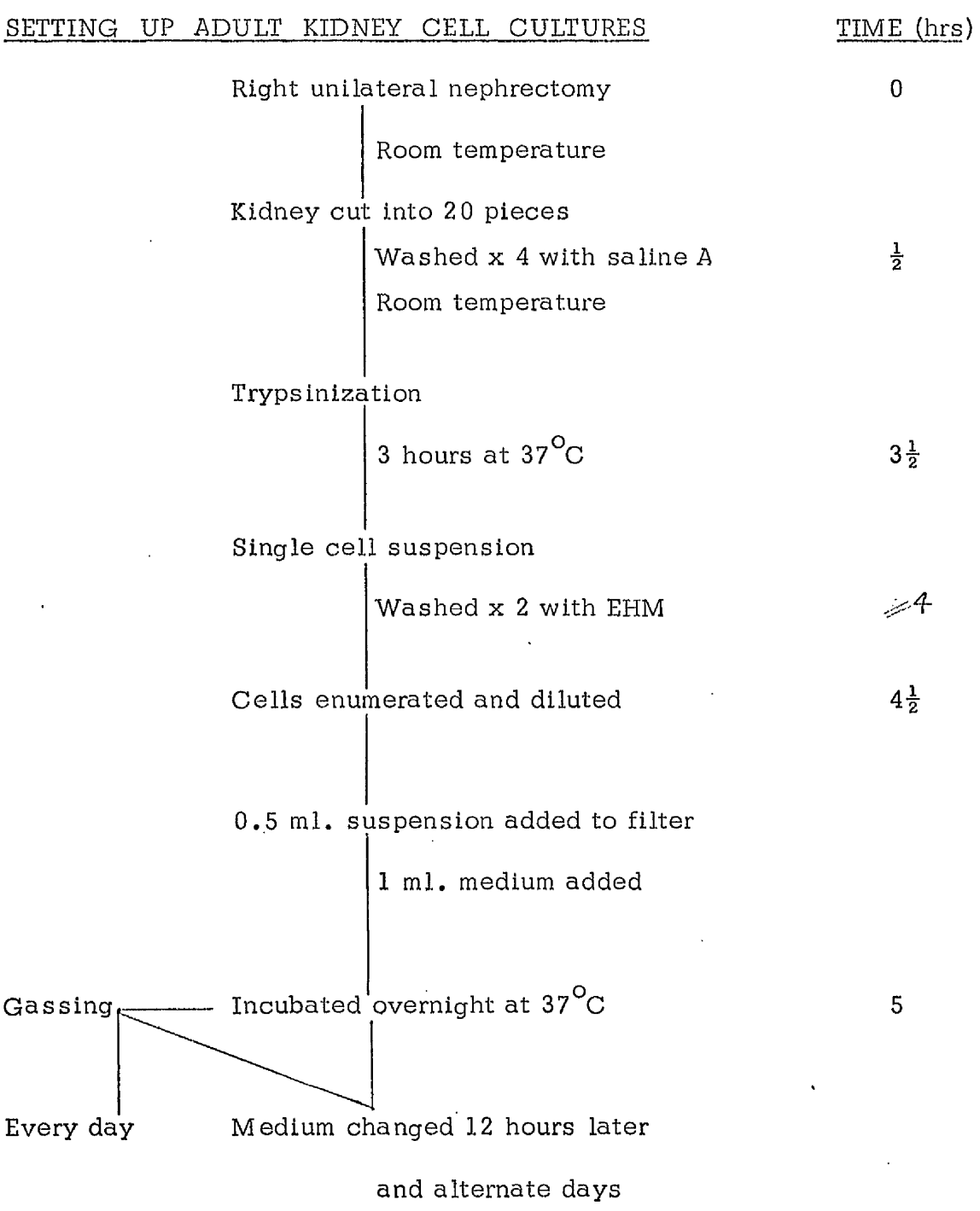
COMPOSITION OF EAGLES MEDIUM

	mg/1000 ml
L-Arginine	105
L-Cysteine	24
L-Histidine	31
L-Isoleucine	52
L-Leucine	52
L-Lysine	58
L-Methionine	15
L-Phenylalanine	32
L-Threonine	48
L-Tryptophan	10
L-Tyrosine	36
L-Valine	46
L-Glutamine	292
Choline	1
Nicotinic Acid	1
Pantothenic Acid	1
Pyridoxal	1
Riboflavine	0.1
Thiamine	1
Inositol	2
Folic Acid	1
Glucose	2000
Na Cl	8000
K Cl	400
$Ca\ Cl_2$	140
$Mg\ SO_4.7H_2O$	100
$Mg\ Cl_2.6H_2O$	100
$Na_2H\ PO_4.2H_2O$	60
$K\ H_2\ PO_4$	60
$Na\ HCO_3$	380
Phenol Red	20
Penicillin	0.5

2.5.2 Method

A young adult male rat weighing 120 g. was used. After right unilateral nephrectomy, the kidney was cut into 20 or so small pieces and washed free of blood with 4 changes of Ca^{2+} and Mg^{2+}-free saline in the 100 ml. conical flask. About 100 ml. of 1% trypsin in saline A (Rinaldini, 1959) was added and disaggregation produced by gentle stirring on a magnetic stirrer in a room at 37°C. It was found that 3 hours of this continuous trypsinization was sufficient to produce complete disaggregation of the tissue. The supernatant was carefully poured into 50 ml. stoppered sterile centrifuge tubes and spun at 500 g. for 10 minutes at room temperature. The layers of cells were then washed twice with 20 ml. of modified Eagles Medium supplemented with 5% calf serum and .1 u insulin/ml. and the cells enumerated on a haemocytomer. The cell suspension was then diluted so that 0.5 ml. contained the number of cells required in the experiment and the cells allowed to settle on the membrane. 1 ml. of medium was added to the vessel so that when the medium above the filter drained through the meniscus would just touch the inferior aspect of the millipore membrane. The filter vessels were then gassed with 95% air and 5% CO_2 until the medium lost any degree of purple it might have; the optimum pH was indicated when the medium became a nice light red colour. The vessels were then incubated in an oven at 37°C overnight. The medium was changed in the morning and on alternate days; gassing was necessary every day. A flow diagram summarizing the process is given in Fig. 3.

FIG. 3


SETTING UP ADULT KIDNEY CELL CULTURES
TIME (hrs)
Right unilateral nephrectomy
0
Room temperature
Kidney cut into 20 pieces
Washed x 4 with saline A
½
Room temperature
Trypsinization
3 hours at 37°C
3½
Single cell suspension
Washed x 2 with EHM
4
Cells enumerated and diluted
4½
0.5 ml. suspension added to filter
1 ml. medium added
Gassing
Incubated overnight at 37°C
5
Every day
Medium changed 12 hours later
and alternate days

Incubation with Isotope

After the standard medium containing 5% calf serum was changed to a medium containing 15% control or nephrectomized rat plasma one hour was left for stabilization to occur. Then isotope was added and the cells gassed. A 6-hour incorporation was used, at the end of which time the micropore filters and attached cells were harvested.

2.5.3 Harvesting of Cells and Preparation for Counting

At the end of the desired period of incubation with isotope, the filter well was removed from the oven and the millipore membrane was eased off the glass ring using a scalpel blade. The membrane and attached cells were transferred to a 15 ml. conical centrifuge tube, the open end covered, and stored at -10°C until all the cultures were harvested.

Reagents

A. 10% TCA

B. 100% TCA

C. 1% potassium acetate in absolute ethanol

D. 0.3 N NaOH

E. Ethanol : Ether - 2 : 1 by volume

F. Hyamine hydroxide

G. Toluene based scintillator containing PPO
(1, 4, Di-(2-(-5 phenyloxazolyl)-benzene))

5 ml. 10% TCA at 0°C were added to the tubes containing the micropore filters and attached tissues, the tubes vigorously shaken

and then allowed to stand at 0°C for 10 minutes. The tubes were centrifuged for 10 minutes at 1000 g to precipitate the denatured nucleic acid and protein, the supernatant discarded and the washing and centrifugation repeated twice. The tubes were drained by inversion and the sides wiped. 2 ml. 0.3 N NaOH were added and the tubes incubated at 37°C for 1 hour. Now the filters were removed from the tubes. Precipitation was produced by the addition of sufficient 100% TCA to make a final concentration of 10% TCA and the tubes centrifuged at 1000 g for 10 minutes at 0°C. The tubes were again drained and wiped, then washed with 5 ml. potassium acetate in ethanol, then with 5 ml. ethanol/ether. Finally the precipitate was dissolved by adding 0.3 ml. hyamine hydroxide and standing overnight at room temperature. The tubes were washed into counting vials using sufficient toluene to make a total volume of 10 ml. After standing overnight at 4°C, the vials were counted in a Phillips Liquid Scintillation Counter.

A flow sheet summarising the procedure is shown in Fig. 4

FIG. 4

PREPARATION OF MATERIAL FOR COUNTING

Tissue and Filter

10% TCA
0°C
1000 g 10'

Supernatant
Discard

Precipitate and Filter

10% TCA
0°C
1000 g 10'

S/N
Discard

Precipitate and Filter

10% TCA
0°C
1000 g 10'

S/N
Discard

Precipitate and Filter

2 ml. 0.3 N NaOH
37°C
1 hour

Filter discarded

ml 100% TCA
Stand 10' at 0°C
Centrifuge at 1000 g for
10' at 0°C

S/N
Discard

Precipitate

1% K Ac. in E t OH
1000 g 10'

S/N
Discard

Precipitate

E t OH : Et_2O 2 : 1
1000 g 10'

S/N
Discard

Precipitate

0.3 ml. Hyamine H Cl
Overnight at room temperature
or 60°C for 20'

Make to 10 ml. with Toluene PPO

2.6 PREPARATION OF HISTOLOGICAL MATERIAL

By trial and error it was found that the simpler the schedule the more chance there was of having some material left at the end of it. The schedule used is given in Table 11. The filter and attached tissue were treated as a unit. At the end of the staining schedule, the filter was placed on a glass slide and cleared by the addition of ethyl acetate or mounting fluid.

TABLE 11

SCHEDULE FOR FIXING, DEHYDRATING AND STAINING TISSUE

10% formol saline	12 hours
Wash in water	½ hour
50% alcohol	½ hour
70% alcohol	½ hour
96% alcohol	½ hour
Absolute alcohol	½ hour
Xylol	6 mins (change once)
Absolute alcohol	6 mins (change once)
Methylated spirits	3 mins
Water	2 mins
Iodine	2 mins
Hypo	2 mins
Water	2 mins
Haemalum	2 mins
Water	Rinse
Acid alcohol	1 min
Water	2 mins
Ammonia water	Until blue
Water	2 mins
Eosin	1 min
Water	Rinse
Absolute alcohol	1 min
Xylol	2 mins

S E C T I O N 3

R E S U L T S

3.1 THE NORMAL RAT KIDNEY WEIGHT

THE RELATIONSHIPS OF KIDNEY WEIGHT TO OTHER PARAMETERS

It was decided to perform a simple investigation to determine if kidney weight is related to the weight of the animal or to the weight of the liver. In order to minimise any variation in weight due to the intake of food and water, 10 animals varying in weight from 160-210 g were caged individually for 7 days before sacrifice. They were given food and water ad libitum until 60 minutes before sacrifice when food and water were removed.

The animals were weighed and sacrificed, the kidneys and livers removed, washed, blotted and weighed on watch-glasses. The dry weights of the tissues were now estimated as described in Section 2.1.4.7. The results are shown in Table 12.

It is clear from these figures that the correlation between total renal weight and rat body weight is not good; a better correlation exists between renal dry weight and rat body weight, and the relationship between renal dry weight and total renal weight is fairly constant. The correlation between liver weight and body weight is fairly good as is that between liver dry weight and body weight. The best correlation is that between kidney dry weight and liver weight, which is better than either the correlation between kidney dry weight and liver dry weight or between kidney dry weight and liver water content.

TABLE 12

$\frac{\text{Total Renal Wt (mg)}}{\text{Rat Body Wt (g)}}$	$\frac{\text{Renal Dry Wt (mg)}}{\text{Rat Body Wt (g)}}$	$\frac{\text{Renal Water Content (mg)}}{\text{Rat Body Wt (g)}}$	$\frac{\text{Renal Dry Wt (mg)}}{\text{Total Renal Wt (mg)}}$	$\frac{\text{Renal Dry Wt (mg)}}{\text{Renal Water Content (mg)}}$
9.487 $\pm$ 3.899	1.977 $\pm$ 0.1222	7.51 $\pm$ 0.71	0.2078 $\pm$ 0.0089	0.2631 $\pm$ 0.0100
$\frac{\text{Liver Wt (mg)}}{\text{Rat Body Wt (g)}}$	$\frac{\text{Liver Dry Wt (mg)}}{\text{Rat Body Wt (g)}}$	$\frac{\text{Liver Water Content (mg)}}{\text{Rat Body Wt (g)}}$	$\frac{\text{Liver Dry Wt (mg)}}{\text{Liver Wt (mg)}}$	$\frac{\text{Liver Dry Wt (mg)}}{\text{Liver Water Content (mg)}}$
54.03 $\pm$ 7.42	13.106 $\pm$ 0.871	40.86 $\pm$ 2.86	0.2398 $\pm$ 0.0080	0.3210 $\pm$ 0.0100
$\frac{\text{Total Renal Wt (mg)}}{\text{Liver Wt (g)}}$	$\frac{\text{Renal Dry Wt (mg)}}{\text{Liver Dry Wt (mg)}}$	$\frac{\text{Renal Water Content (mg)}}{\text{Liver Water Content (mg)}}$	$\frac{\text{Renal Dry Wt (mg)}}{\text{Liver Wt (mg)}}$	$\frac{\text{Renal Dry Wt (mg)}}{\text{Liver Water Content (mg)}}$
0.1748 $\pm$ 0.0202	0.1522 $\pm$ 0.0100	0.1838 $\pm$ 0.0200	0.0365 $\pm$ 0.0002	0.0483 $\pm$ 0.0046
$\frac{\text{Renal Water Content (mg)}}{\text{Liver Wt (mg)}}$	$\frac{\text{Renal Water Content (mg)}}{\text{Liver Dry Wt (mg)}}$			
0.1196 $\pm$ 0.0412	0.5760 $\pm$ 0.0761			

The relationships between various parameters of kidney and liver weight. The values are the means for 12 animals $\pm$ Standard Deviation. Whether or not a correlation is present can be inferred from the magnitude of the Standard Deviation relative to the mean.

3.2 CHANGES OCCURRING IN THE PHYSIOLOGICAL AND BIOCHEMICAL STATE OF THE RAT FOLLOWING UNILATERAL NEPHRECTOMY

In seeking the stimulus to the compensatory growth which occurs following removal of one kidney, it was decided first to study the effect of unilateral nephrectomy on the physiological and biochemical state of the animal. The kidney is concerned in the control of the relative and actual concentrations of sodium and water, the control of acid-base balance and ammonia metabolism, the control of the arterial blood pressure and various other aspects of metabolism, including the synthesis of creatine as well as the excretion of many substances. Consequently, a series of short experiments was planned to study the effect of unilateral nephrectomy on some of these parameters.

3.2.1 The Effect of Unilateral Nephrectomy on Plasma Sodium Levels

Halliburton (1966) found no consistent alteration in serum sodium levels at 24 hours and 48 hours following unilateral nephrectomy in the rat. Borelli et al. (1962), in the dog, report a transient increase in plasma sodium in the first day following unilateral nephrectomy, followed by a slight fall in the next 4 days. They report no change in plasma potassium or chloride levels, but a fall in urinary sodium and chloride in the first 3 days following unilateral nephrectomy and

an increase in urinary potassium. They explain these findings as due to alteration in adrenal activity following unilateral nephrectomy.

Consequently it was decided to study the levels of plasma sodium at various times in the first few days after unilateral nephrectomy in the rat. Six pairs of rats, all weighing around 200 g were used in the experiment. One animal of each pair was nephrectomized and the other had a sham operation. At each time interval chosen, 2 pairs of rats were studied. Plasma sodium levels were estimated before operation and at sacrifice. The results are shown in Table 13. It can be seen from these figures that there is no consistent alteration in plasma sodium levels after unilateral nephrectomy compared to sham-operated animals. A fall in plasma sodium is found in both groups at 16 and 24 hours after operation. This may be due to a reduction in food intake in the first 24 hours after operation.

TABLE 13

Treatment	Plasma Sodium milliequiv/l.		
	Pre-operative Level	Post-operative Level	Time (hours) of Post-operative Reading
Sham Nephrectomy	149[a]	149	6
	144	140	8
	153	130	16
	156	133	24
Right Unilateral Nephrectomy	142	143	6
	124	124	8
	149	127	16
	152	144	24

Change in plasma sodium levels in the rats at various times after sham operation and right unilateral nephrectomy.

a. All values are means for 2 rats weighing 200 g.

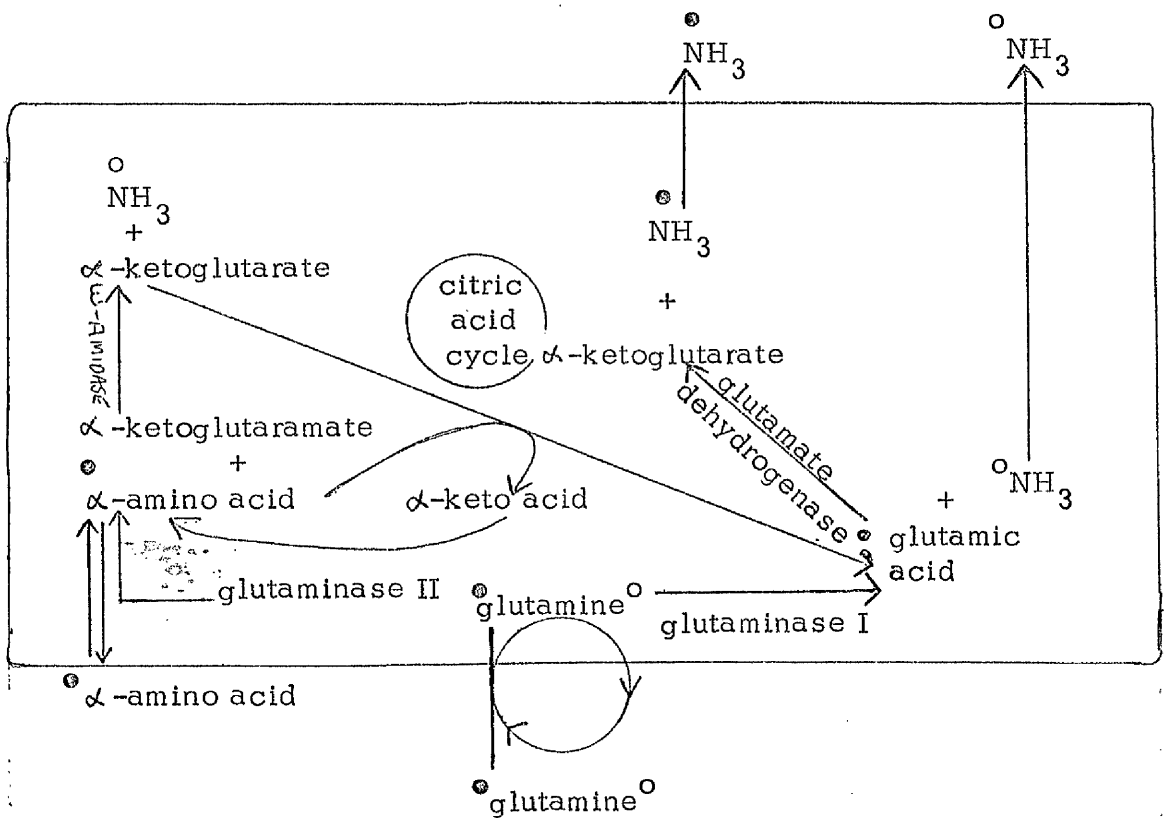
3.2.2 Plasma Amino Acid Analysis

There are several reasons why an investigation of the changes occurring following unilateral nephrectomy should include plasma amino acid estimations. Firstly, it is known that diets high in protein produce renal growth, especially a high gelatin diet (Moore, 1967). Also, a diet high in glycine gives an increase in kidney size (Moore, 1967). It would be interesting to note whether or not unilateral nephrectomy alters the plasma glycine level. Secondly, the kidney plays an important role in the metabolism of ammonia via its role in the maintenance of acid-base balance. The production of ammonia by the kidney is shown in Fig. 5. Pitts (1968) has shown that in dogs 33-50% of ammonia excreted by the kidney comes from the amide nitrogen of glutamine and a further 16-25% from the amino nitrogen of glutamine. Other amino acids, including glycine and alanine, can act as donors in ammonia production (Pitts, 1968).

Amino acid analysis was performed on venous blood from animals nephrectomized at various intervals. The tracings showed only minor changes until the experiment was performed on blood from an animal nephrectomized 14 hours previously; this showed a great fall in the plasma glutamine level and an increase in the glutamic acid level. This experiment has as yet not been repeated and the results cannot be shown here. Also, there appeared to be an increase in blood ammonia on amino acid analysis. Consequently, plasma ammonia was investigated more carefully.

FIG. 5

THE PRODUCTION OF AMMONIA BY RENAL TUBULAR CELLS



● - amino nitrogen

o - amide nitrogen

3.2.3 Blood Ammonia Levels

On the amino acid analysis curves just described there appeared to be some increase in ammonia levels in some of the tracings. However, ammonia estimated in this way is not a true reflection of the blood ammonia level at the time of withdrawal of blood (Leaf, 1969). Consequently it was decided to investigate the blood ammonia level in normal and nephrectomized rats by a more sensitive technique, using the enzyme glutamate dehydrogenase. The methods is described in detail in Section 2.2.6.

Four pairs of animals were used in the experiment. Under ether anaesthesia 2 ml. of venous blood were withdrawn from the left jugular vein into a heparinised syringe; the animal was then either nephrectomized or sham operated. One pair of animals was again anaesthetised at the end of 4 hours and 2 ml. of blood withdrawn from the right jugular vein, the animals were then sacrificed. A further 2 pairs were sacrificed 14 hours post-operatively, again after withdrawal of blood from the jugular vein, and the last pair were sacrificed 24 hours after unilateral nephrectomy. Two pairs of animals were chosen at 14 hours after nephrectomy since it was at this time that the change in glutamate/glutamine ratio was most apparent on amino acid analysis and the apparent increase in blood ammonia was noted at this time.

Ammonia estimations were performed on the blood samples as described in Section 2. The results are shown in Table 14.

TABLE 14

Treatment	Blood Ammonia µg/100 ml		Post-operative Time of Second Reading (hours)
	First Reading	Second Reading	
Sham Operation	95.8[a]	59.4	4
Unilateral Nephrectomy	71.4	42.5	4
Sham Operation	44.4	81.0	14
" "	68.4	60.2	14
Unilateral Nephrectomy	57.9	7.7	14
" "	77.2	52.0	14
Sham Operation	34.8	32.8	24
Unilateral Nephrectomy	32.7	69.5	24

Blood ammonia levels at various times after sham operation and right unilateral nephrectomy.

a. Each reading represents one rat weighing around 200 g.

These results show that the blood ammonia level does change both in sham operated and nephrectomized animals, but there is no constancy in the direction of these changes. What is clear is that the apparent increase in blood ammonia levels seen on amino acid analysis is certainly an artefact and there is no reason to justify a more prolonged study of blood ammonia following unilateral nephrectomy.

3.2.4 Acid-Base Balance

Two important functions of the kidney are the excretion of acid and the buffering of urine. It is possible therefore that removal of one kidney results in some upset of acid-base balance. Estimations of acid-base balance were performed on Micro Astrup equipment as described in Section 2.2.5. This is something which is obviously much more easily and accurately performed on an animal larger than the rat since it involves obtaining arterial or capillary blood. It is necessary to anaesthetise a rat in order to obtain arterial blood, and this was in fact done, using ether.

Blood from the rat aorta was obtained as described in Section 2.1.4.5. Since most animals bled fairly profusely following puncture of the aorta, it was not possible to perform pre- and post-nephrectomy estimations on the same animals. Ten animals, weighing around 200 gm. were used. Two animals were anaesthetised with ether and blood withdrawn from the aorta and the acid-base balance estimated as described. Of the other 8 animals, 4 were nephrectomised and 4 sham operated. Blood was withdrawn from 2 nephrectomized and 2 sham operated animals 16 hours later and the remaining 4 animals were similarly treated 24 hours after unilateral nephrectomy. The results are shown in Table 15.

TABLE 15

Treatment	Time after Treatment of Aortic Puncture (hrs)	pH	Actual pCO_2	Base Excess m.eq/l	Buffer Base m.eq/l	Standard Bicarbonate m.eq/l
None	0	7.38[a]	42.5	0	51	24
None	0	7.40	40.0	0	47	24
Sham	16	7.60	10	1	61.5	24.5
Sham	16	7.58	10	0	61	24.5
Unilateral Nephrectomy	16	7.60	10	-11	46.5	17
Unilateral Nephrectomy	16	7.62	21.5	+18	47	17
Sham	24	7.43	30	-1	56	23.5
Sham	24	7.45	33	+1	57	25
Unilateral Nephrectomy	24	7.48	32.5	+2.5	54.5	26.5
Unilateral Nephrectomy	24	7.49	26	0	54	24.5

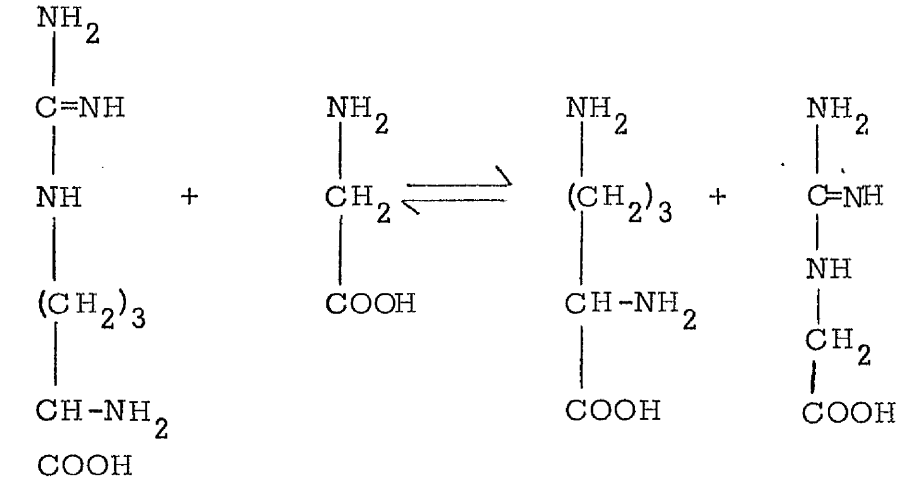
Acid-base balance at various times after sham operation and right unilateral nephrectomy.

a. Each set of readings represents one rat weighing around 200 g.

It can be seen from these results that operation, whether a unilateral nephrectomy or a sham operation produces a rise in pH and a fall in pCO_2 at 16 hours; this has returned to normal by 24 hours and is presumably due to post-operative hyperventilation by the rat.

3.2.5 Amidotransferase (2.6.2.1) Activity

The various methods used to estimate the degree of compensatory growth occurring in the solitary kidney of the unilaterally nephrectomized animal were given in the Introductory Section. Little attempt has been made to determine whether or not the growth which has been estimated as increased mitotic index, DNA, RNA or protein content, is translated into an increase in specific biochemical components of renal tissue. From this point of view it was decided to study the enzyme amidotransferase. This enzyme is involved in the first stage of creatine synthesis, i.e. the formation of guanido - acetic acid and ornithine from arginine and glycine :



The second reaction in the synthesis, i.e. the reaction of guanido-acetic acid and methionine to form homocysteine and creatine occurs in the liver, but the first stage, in the rat at least, occurs almost exclusively in the kidneys (Van Pilsum et al., 1967a).

The enzyme has attracted some interest since the discovery by Walker (1959 and 1961) that the addition of 2% creatine to the diet reduces the amidotransferase activity of rat kidneys or chick livers to very low levels. He also found that the levels were independent of plasma or kidney creatinine levels. Van Pilsum et al. (1967a) on the other hand found that rat kidney amidotransferase activity was related to the level of creatine in the plasma or kidneys of protein-depleted or starving rats, being lower when the plasma creatine became elevated. Van Pilsum et al. (1967b) also reported an increase in rat total carcass amidotransferase activity, after intraperitoneal injection of arginine plus glycine; by consideration of the results in Section 3, it seems possible that Van Pilsum induced a degree of renal hypertrophy by injection of glycine and that this and not any direct effect on the enzyme explains the increase in amidotransferase he obtained. However, it would be interesting to determine what happens to kidney amidotransferase activity in the early stages following unilateral nephrectomy.

Nine male rats weighing approximately 200 g. were used. Three animals were nephrectomized and 3 sham-operated. Three were unoperated controls. Forty-eight hours later the animals were

sacrificed and the kidneys removed. Amidotransferase was assayed as described in Section 2.4; all assays were performed at the same time. The results are expressed in optical density units and are shown in Table 16.

It can be seen from these results that unilateral nephrectomy does not produce an increase in the amidotransferase activity of the remaining kidney. At first sight, the animals which had a sham operation would appear to have lower amidotransferase activity than either of the other 2 groups. Statistical evaluation by student t testing does not support this, even in the case of the largest difference between groups (a in Table 16). This might, however, be due to the fact that the numbers of rats in each group (3) is too small, since student t testing reveals only large differences between small groups. The results do, however, give ground for suspicion that sham-operation depresses the total amidotransferase activity and that this depression is overcome in the kidney remaining after unilateral nephrectomy. If this is the case, it would correlate well with the findings of Van Pilsum (1967a) that kidney amidotransferase activity is depressed when plasma creatine is high as operation is likely to have an effect similar to starvation in liberating creatine from muscle.

TABLE 16

Treatment	No. of Animals	Kidney Amidotransferase Activity (OD Units)		Activity per 100 mg Tissue (OD Units)		Left/Right Ratio
		Left	Right	Left	Right	
None	3	3.61 ± 0.60	4.11 ± 0.14	0.605 ± 0.059	0.642 ± 0.033	0.900 ± 0.083
Sham Nephrectomy	3	2.75 ± 0.59 [a]	3.22 ± 0.43	0.451 ± 0.040	0.471 ± 0.056	0.866 ± 0.110
Right Unilateral Nephrectomy	3	5.84 ± 1.86	4.43 ± 1.53	0.801 ± 0.140	0.633 ± 0.180	1.482 ± 0.335

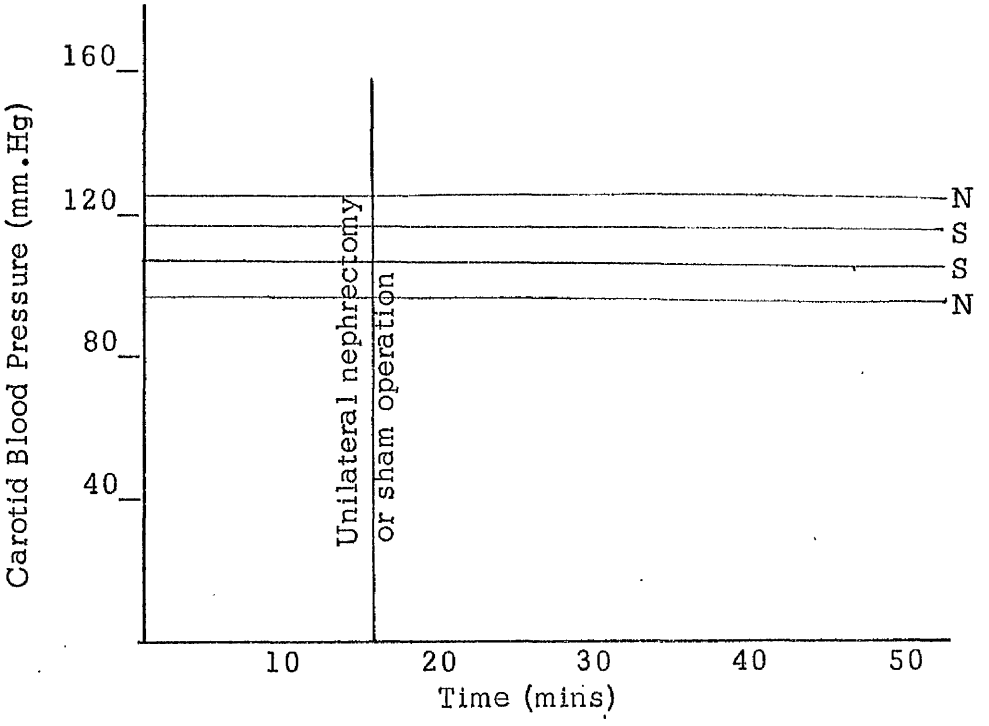
Kidney amidotransferase activity in normal, sham-operated and right unilaterally nephrectomized rats, weighing around 200 g.

a. Difference not statistically significant ($p > 0.05$)

3.2.6 Arterial Blood Pressure

The kidney plays an essential role in the maintenance of arterial blood pressure. The main theories on the mechanism by which it does this are given in Section 3.5.2. Four animals were used to determine whether unilateral nephrectomy has any effect on arterial blood pressure level. Blood pressure was estimated by means of carotid canulation as described in Section 2.1.4.4. The animals were anaesthetised with pentobarbitone as described in Section 2.1.4.4. Blood pressure readings were taken at intervals for 15 minutes before unilateral nephrectomy, and for 30 minutes afterwards. The results are shown in Fig. 6. It is clear that unilateral nephrectomy has no effect on rat arterial blood pressure, at least in the first 30 minutes.

FIG. 6 : The Effect of Unilateral Nephrectomy or Sham Operation on Rat Arterial Blood Pressure



N - nephrectomized animal S - sham-operated animal

3.3 THE EFFECT OF ADMINISTRATION OF VARIOUS SUBSTANCES ON KIDNEY GROWTH

The compensatory growth of the kidney which occurs following contralateral nephrectomy has often been claimed to be due to work hypertrophy (cf. Section 1), in the same way as a muscle which is repeatedly exercised responds by hypertrophying. If this is the case, it should be possible to produce hypertrophy and perhaps atrophy of the kidney by dietary means or by injection of substances handled by the kidney. Agents which have previously been shown to affect the growth rate of the kidney were described in the introductory section. The most consistent effect observed has been that of gelatin and glycine in producing renal hypertrophy. The effect of glycine has been studied again in this investigation as well as the effect of other amino acids and inorganic and organic salts, and variation in protein and fat content of the diet.

3.3.1 The Effect of Injection of a Urea/Saline Mixture on Kidney Weight and Composition

Various experiments involving ureteral transplantation have already been described in Section 1; in those cases in which the operative procedures produced growth of the kidney, it was argued that this was due to the extra excretory work which the kidneys had to perform. It was decided to perform a series of experiments involving the repeated injection of a hypertonic solution of urea and saline.

This work has already been reported by Thomson (1970). The results are included in this work also and are shown in Tables 17 to 22.

It is clear that the repeated injection of such a hypertonic solution has no effect on kidney weight or the renal content of RNA. It is also clear that such injections have no effect on the response to unilateral nephrectomy at 96 hours (Table 22).

TABLE 17

Treatment	No. of Animals	Mean Weight Gain (g)	Total Renal Weight[b] (g)
Injected with 20 ml urea/saline mixture over 8 days	3[a]	19	1.55 ± 0.137
Anaesthetised but uninjected controls	6	17.5	1.55 ± 0.130

The effect on body weight and total renal weight of the intravenous injection of 20 ml. of urea/saline mixture over 8 days.

a. The other 3 animals in this group died during the injections and were excluded.

b. Values are the means for the group ± Standard Error.

TABLE 18

Treatment	No. of Animals	Mean Weight Gain (g)	Total Renal Weight[a] (g)
Injected with 30 ml urea/saline mixture over 7 days	6	-4	1.98 ± 0.220
Anaesthetised but uninjected controls	6	8	2.14 ± 0.124

The effect on body weight and total renal weight of the intravenous injection of 30 ml. of urea/saline mixture over 7 days.

a. Values are the means for the group ± Standard Error.

TABLE 19

Treatment	No. of Animals	Mean Weight Gain (g)	Total Renal Weight[a] (g)
Injected with 25 ml urea/saline mixture over 5 days	5	-16	1.73 ± 0.064
Anaesthetised but uninjected controls	6	11	1.91 ± 0.145

The effect on body weight and total renal weight of the intravenous injection of 25 ml. of urea/saline mixture over 5 days.

a. Values are the means for the groups ± Standard Error.

TABLE 20

Treatment	No. of Animals	Mean Weight Gain (g)	Total Renal Weight[b] (g)
Injected with 25 ml urea/saline mixture over 5 days	7[a]	3	1.49 ± 0.152
Anaesthetised but uninjected controls	12	7	1.57 ± 0.173

The effect on body weight and total renal weight of the intravenous injection of 25 ml. of urea/saline mixture over 5 days.

a. 5 animals in this group died during injection and were excluded.

b. Values are the means for the groups ± Standard Error.

TABLE 21

Treatment	No. of Animals	Mean Weight Gain(g)	Total Renal Weight (g)[a]	Total Renal RNAP µg[a]	µg RNAP/ 100 mg Tissue[a]
Injected with 25 ml urea/ saline mixture over 4 days	6	-11	1.45 ± 0.122	705 ± 20	48.9 ± 3.29
Anaesthetised uninjected controls	12	-7	1.43 ± 0.134	689 ± 39	48.3 ± 3.64

The effect on body weight, total renal weight and total renal RNAP of the intravenous injection of 25 ml. of urea/saline mixture over 4 days.

a. Values are means for the groups ± Standard Error.

TABLE 22

Treatment	No. of Animals	Mean Weight Gain(g)	Right Kidney Weight (g)	Left Kidney Weight (g)	Lt/Rt Ratio
Right unilateral nephrectomy + injection of 10 ml urea/saline mixture over 4 days	4	-9	0.670 ± 0.027[a]	0.787 ± 0.018[b]	1.15 ± 0.020
Right unilateral nephrectomy + anaesthetised not injected	6	1	0.730 ± 0.031	0.855 ± 0.041[c]	1.17 ± 0.02

The effect on the response to right unilateral nephrectomy of the injection of 16 ml. of urea/saline mixture in the 96 hours between operation and sacrifice.

a. Values are means for the groups ± Standard Error.

b. Change significantly different from zero ($p < 0.05$)

c. Change significantly different from zero ($p < 0.05$)

3.3.2 The Effect of Diets containing Sodium Chloride and Potassium Chloride

Since much of the kidney's work is performed in the absorption of sodium, it might be reasonable to suppose that feeding a diet high in sodium might cause renal growth. An essential function of the kidney is the excretion of potassium which is toxic in excess; therefore it might be reasonable to suppose that a diet high in potassium might result in growth of the potassium excretory apparatus. It has been shown that in dogs given high doses of potassium chloride for several days adaptation occurs so that the excess potassium is excreted more rapidly as time goes on (Pitts, 1968). This suggests induction of an enzyme system and it might be possible that in a small animal such as the rat any increase in enzyme content might be measured as growth.

Eighteen male rats weighing around 200 g. were used in the experiment, and divided into 3 groups. One group was fed a control stock diet, another a stock diet supplemented with 2% by weight of Na Cl and the third group were fed a stock diet supplemented with sufficient K Cl to make it iso-osmolar with 2% Na Cl. The composition of the diets is given in Table 8. At the end of 6 days the animals were sacrificed and the kidneys and livers removed and weighed. The results are shown in Table 23. It is clear from these results that the supplementation of the rat diet with 2% Na Cl or an iso-osmolar quantity of K Cl has no effect on kidney weight or liver weight.

TABLE 23

Treatment	No. of Animals	Mean Final Body Weight (g)	Total Renal Wt (g)	Liver Weight (g)	$\frac{\text{Liver Wt (mg)}}{\text{Final Body Wt (g)}}$	$\frac{\text{Renal Wt (mg)}}{\text{Final Body Wt (g)}}$	$\frac{\text{Liver Wt (g)}}{\text{Kidney Wt (g)}}$
Control Diet	5	164	1.372 ± 0.081[a]	6.71	40.31 ± 5.68	8.26 ± 0.52	4.88 ± 0.66
High NaCl Diet	6	168	1.350 ± 0.069	5.96	36.57 ± 3.46	8.01 ± 0.44	4.55 ± 0.57
High KCl Diet	6	168	1.335 ± 0.148	6.41	38.07 ± 4.40	7.95 ± 0.53	4.80 ± 0.37

The effects of diets supplemented with NaCl and KCl on body weight and kidney and liver size.

a. Values are means for groups $\pm$ S.D.

3.3.3 The Effects of Dietary Gelatin and Glycine on Kidney Weight and Composition

Moore (1967) found that a diet containing 30% gelatin produced a 50% increase in renal weight in 6 days as well as a 25% increase in RNA/DNA, i.e. it had an effect similar to unilateral nephrectomy. Now, a diet containing 30% gelatin represents a diet containing 8% glycine, since glycine is the most abundant amino acid in gelatin (Moore, 1967). A diet containing 8% glycine produced a degree of renal hypertrophy, to the extent of 25% increase in renal mass, and an increase in RNA/DNA and an increase in protein/DNA. There was no increase in DNA. In the present investigation, these experiments were repeated and in addition the effect on the liver was noted.

Twelve male rats, 6 control and 6 test, were used in the experiment on gelatin. One group was fed a 30% gelatin diet for 6 days, the other was fed the usual control diet. At the end of 6 days, the animals were sacrificed and the kidneys and livers removed, weighed and analysed for RNA, DNA and protein. The results are shown in Tables 24-26. It can be seen from these figures that a diet containing 15% casein and 30% gelatin gives a 40% increase in renal weight over 6 days compared to the control diet which contains 15% casein. It gives a 25% increase in renal RNA, a 22% increase in protein and a 7% increase in DNA, though this increase in DNA is not statistically significantly different from zero.

This can be compared to the findings of Halliburton (1966) of the growth response obtained in compensatory renal hypertrophy. A 25% increase in renal RNA occurs at 48 hours after unilateral nephrectomy as does a 22% increase in renal protein and a 7% increase in renal DNA. The amount of RNA/cell and protein/cell shows an 18% increase, which is of similar extent to that occurring following unilateral nephrectomy. However, the increase in renal weight (40%) occurring in animals fed the gelatin is double that which would be expected to occur in unilaterally nephrectomized animals at 48 hours. Also, in compensatory renal hypertrophy, the RNA concentration, i.e. RNA/100 mg. tissue shows approximately a 12% increase at 48 hours as does the protein/100 mg. ratio whereas in animals fed 30% gelatin for 6 days there is an 8% reduction in RNA/100 mg. and a 7% reduction in protein/100 mg. The mean cell mass is inversely proportional to the content of DNA/100 mg. At 48 hours after unilateral nephrectomy, the mean cell mass does not increase whereas there is a 22% increase in mean cell mass in animals fed gelatin for 6 days. In other words, the process occurring when a high gelatin diet is fed is identical to that occurring in compensatory renal hypertrophy, but in addition some other substance besides DNA, RNA and protein accumulates in the cell.

TABLE 24

Treatment	No.of Animals	Mean Wt. Gain (g)	Total Renal Wt (g)	Total Renal RNAP(µg)	RNAP(ug) / 100 mg	Total Renal Protein mg	Protein mg / 100 mg	Total Renal DNA µg	DNA µg / 100 mg	RNAP µg / DNA µg	Protein mg / DNA µg
Control Diet 15% Casein	6	21 ±	1.250 ± 0.14[a] ↑ b	651 ± 94 ↑ c	52 ± 9.0	210±33 ↑ d	17 ± 2.8	5880±740	460±78 ↑ e	0.11±0.037 ↑ f	0.037±0.005 ↑ g
High Protein Diet 15% Casein+ 30% Gelatin	6	19	1.760 ± 0.02 ↓	815 ± 81 ↓	48 ± 6.6	256±60 ↓	16 ± 2.0	6270±480	360±32 ↓	0.13±0.014 ↓	0.044±0.004 ↓

The effect of a diet containing 30% gelatin compared to a control diet containing 15% casein on kidney weight and composition.

a. Values are means for the groups ± S.D.

b. Difference highly significantly different from zero ($p < 0.001$) : c. Difference significantly different from zero ($p < 0.01$)

d. Difference significantly different from zero ($p < 0.025$) : e. Difference significantly different from zero ($p < 0.01$)

f. Difference significantly different from zero ($p < 0.01$) : g. Difference significantly different from zero ($p < 0.05$)

TABLE 25

	Control Diet containing 15% Casein	High Protein Diet containing 15% Casein + 30% Gelatin
No. of Animals	6	6
Mean Body Weight Gain (g)	21	19
Liver Weight (g)	7.67 ± 1.35	8.47 ± 0.96
Total Liver RNAP μg	6410 ± 734	6680 ± 800
$\frac{\mu g \text{ RNAP}}{100 \text{ mg}}$	82.3 ± 16	79.3 ± 9.8
Total Liver Protein	1670 ± 315	1940 ± 160
$\frac{\mu g \text{ Protein}}{100 \text{ mg}}$	21.4 ± 2.3	22.4 ± 1.45
Total Liver DNA μg	22950	23310
$\frac{\mu g \text{ DNA}}{100 \text{ mg}}$	256	256
$\frac{\mu g \text{ RNAP}}{\mu g \text{ DNA}}$	0.26	0.30

The effect of a diet containing 30% gelatin compared to a control diet containing 15% casein on liver weight and composition.

a. Values are means for the groups ± S.D.

TABLE 26

The Effect of a Diet containing 30% Gelatin on Kidney and Liver Weights and Compositions

	% Increase or Decrease due to Gelatin over 15% Casein Diet
Body weight gain	0
Total renal weight	41
Total renal RNAP	25
Renal RNAP/100 mg tissue	-8*
Total renal protein	22
Renal protein/100 mg tissue	-7*
Total renal DNA	7
Renal DNA/100 mg tissue	-18
Liver weight	9**
Liver RNAP	4**
Liver RNAP/100 mg	-4**
Liver protein	17**
Liver protein/100 mg	23
Liver DNA	0

* = not significantly different from zero

** = not significant

Gelatin is a diuretic and is known to increase the GFR (Vogl, 1953), and it may be that the substance accumulating in the kidney is water. The growth of the kidney induced by gelatin might be related to this phenomenon.

In the case of the liver (see Tables 24 and 25), there is no statistically significant difference in liver weight or composition. There is a 17% increase in liver protein content, but this is not supported as a positive finding by student t test analysis.

Glycine is the most abundant amino acid in gelatin and a diet containing 8% glycine is equivalent to one containing 30% gelatin as far as glycine content is concerned (Moore, 1967). Two groups of 6 animals were fed a diet containing 8% glycine and a control diet respectively for 6 days and the kidneys and livers removed and analysed as before. The composition of the diets is shown in Table 7.

The results are shown in Tables 27 to 29, and the results of a subsequent experiment are shown in Table 30.

TABLE 27

Treatment	No. of Animals	Mean Weight Gain (g)	Total Renal Wt (g)	Liver Weight (g)	Renal Weight / Body Weight (mg/g) [e]
Control Diet	5[a]	19	1.213 ± 0.123[b] [c]	8.290 ± 0.289	6.728±0.574 [d]
Diet containing 8% glycine	6	21	1.382 ± 0.066	8.456 ± 1.183	7.692±0.490

The effect of a diet containing 8% glycine compared to a control diet on kidney and liver weights.

a. One animal from this group showed persistent severe weight loss and was excluded.

b. Values are means for the groups ± S.D.

c. Difference significantly different from zero ($p < 0.01$)

d. Difference significantly different from zero ($p < 0.01$)

e. The ratio $\frac{\text{renal weight}}{\text{body weight}}$ is given here for purposes of comparison with later experiments.

TABLE 28

Treatment	No. of Animals	RNAP/Kidney (µg)		RNAP/100 mg		Mean RNAP/DNA	Total Kidney RNAP / Final Body Weight
		Left	Right	Left	Right		
Control Diet 15% Casein	5	233 ± 19.6[a]	240 ± 24.8	39.4 ± 1.2	38.6 ± 0.4	0.0909 ± 0.0100	2.73 ± 0.37
		f	b	c		d	e
Diet containing 8% Glycine	6	252 ± 42.0	251 ± 40.5	37.2 ± 5.9	36.0 ± 6.3	0.1039 ± 0.0203	2.82 ± 0.45

The effect of a diet containing 8% glycine on kidney RNAP, compared to a control diet containing 15% casein. The values represent analyses of the kidneys from the animals in Table 27.

a. Values are means for the groups ± S.D.

Statistical analyses of b (difference between total RNAP in the 2 groups), c (difference between mean RNAP/100 mg in the 2 groups), d and e show no significant difference between the 2 groups if performed by Student t test.

c.f. Analysis of Variance performed by the technique of R. A. Fisher (Moroney, 1951) (see Table 37 and Fig. 9) gives F value of 7.5, i.e. $p < 0.05$ and p almost = 0.01. Therefore the difference between left kidney RNA is significantly different from zero.

TABLE 29

Treatment	No. of Animals	Protein/Kidney mg		Protein/100 mg		DNA/Kidney (ug)		Mean DNA/100 mg (ug)
		Left	Right	Left	Right	Left	Right	
Control Diet 15% Casein	5	112 ± 20[a]	122 ± 19	18.9 ± 2.2	19.7 ± 2.4	2676 ± 583	2597 ± 458	436 ± 48 ↑ b
Diet containing 8% Glycine	6	126 ± 10	116 ± 12	18.3 ± 1.3	16.6 ± 1.7	2564 ± 190	2242 ± 188	346 ± 28 ↓

The effect of a diet containing 8% glycine on kidney composition. The values represent analyses of kidneys from animals in Table 27.

a. Values are means for the groups ± S.D.

b. Difference significantly different from zero ($p < 0.01$)

TABLE 30

Treatment	No. of Animals	Mean Weight Gain (g)	Total Renal Wt (g)	Renal Weight / Body Weight
Control Diet	5	20	1.110 ± 0.167[a]	6.953 ± 0.346
			b	c
Diet containing 8% glycine	5	18	1.285 ± 0.066	8.233 ± 0.827

The effect of a diet containing 8% glycine on kidney weight.

a. Values are means for the groups ± S.D.

b. Difference significantly different from zero ($p < 0.05$)

c. Difference significantly different from zero ($p < 0.05$ and almost $= 0.01$).

It can be seen from these results that the diet containing 8% glycine caused a 14% increase in renal weight in the first experiment (Tables 28 and 29) and a 16% increase in renal weight in the second experiment (Table 30). These results are statistically significant. There was a 10% increase in RNA and a 14% increase in RNAP/DNA. However, these changes do not withstand statistical test by the method of student t test. The effect of the addition of potassium citrate was tested at the same time as this experiment (see Section 3.3.5). It was necessary to perform analysis of variance by Snedecor's F test in order to evaluate the results and this showed that the difference in left kidney RNAP is significantly different from zero ($p < 0.05$ and p almost=0.01). Halliburton (1966) showed that the relationship between the RNAP of left and right kidneys is constant and left kidney RNAP may be taken as an index of total kidney RNAP. Therefore, one concludes that glycine produces a 10% increase in kidney RNAP and a 14% increase in RNAP/DNA.

It does not produce any change in the total amounts of protein or DNA. However, the amount of DNA/100 mg is significantly reduced by 21% ($p < 0.01$), i.e. an increase in mean cell mass. These changes, when compared to those produced by gelatin, are seen to be basically similar, i.e. an increase in mean cell mass plus an increase in RNAP (and also protein in the case of gelatin). It may well be that the glycine effect represents an identical, though less intense, stimulus to growth than the gelatin effect.

Moore (1967) obtained a 25% increase in RNAP with an 8% glycine diet. The difference between these figures and Moore's might be due to differences in the animals used or in the glycine; the effect might, of course, be due to some contaminant of the glycine.

In Table 30 the results of the second experiment are shown. In this case there is a 16% increase in total renal weight ($p < 0.05$) and there is an 18% increase in $\frac{\text{Renal weight}}{\text{Body weight}}$ ($p < 0.05$). These results are similar enough to discount the suggestion that an 8% glycine diet produces a significant effect on the rat's body weight.

3.3.4 The Effect of Other Amino Acids on Body Weight, Kidney and Liver Weights

In order to check whether the effects described might occur with any amino acid, the experiments just described were repeated using L-arginine as the amino acid supplement. In fact, both L-arginine, HCl and L-arginine-free base were used in case any response might be attributed to the wrong part of the molecule.

Rats were again used and were fed and sacrificed in the usual manner. The results are shown in Tables 31 and 32. The composition of the diets is shown in Table 8. From Table 31 it can be seen that the diet containing 22% L-arginine-HCl produces a significant increase in renal weight ($p < 0.01$) coupled with a fall in body weight and a highly significant increase in $\frac{\text{kidney weight}}{\text{body weight}}$ ratio ($p < 0.001$). It also produces a highly significant fall in

liver weight ($p < 0.001$) and a significant ($p < 0.05$) fall in $\frac{\text{liver weight}}{\text{body weight}}$ i.e. L-arginine-HCl produces an actual and a relative increase in kidney weight together with an actual and relative decrease in liver weight. The effect is presumably due to chronic metabolic acidosis induced by HCl as L-arginine-free base does not produce an effect of this magnitude.

From Table 32 it can be seen that L-arginine-free base produces a fall in body weight of the order of 8% (not statistically significant) and an increase in renal weight of 6% (not significant). However, there is an increase of 18% in the ratio $\frac{\text{renal weight}}{\text{body weight}}$ and this is highly significant ($p < 0.001$).

It is not clear to what this is due. It is similar to the effect produced by L-arginine-HCl but of a much smaller magnitude. It may be that this quantity of L-arginine is toxic to the rat, causing a fall in body weight, whereas the amount of proteinaceous material ingested produces a slight degree of increase in renal weight due to hypertrophy.

TABLE 31

	Treatment	
	Control Diet	Diet supplemented with L-Arginine-HCl
No. of Animals	5	6
Mean Initial Body Wt	164	161
Final Body Wt	180.000 ± 12.500[a]	132.000 ± 21.0
Total Renal Wt	1.213 ± 0.123 [b]	1.394 ± 0.168
Liver Wt	8.290 ± 0.289 [c]	4.290 ± 0.792
Kidney Wt / Body Wt	6.730 ± 0.570 [d]	10.310 ± 0.870
Liver Wt / Body Wt	46.100 ± 3.680 [e]	35.042 ± 7.937

The effect of a diet supplemented with 22% L-Arginine-HCl on body weight, kidney and liver weights.

a. Values are means for the group ± S.D.

b. Difference significantly different from zero ($p < 0.01$)

c. Difference highly significantly different from zero ($p < 0.001$)

d. Difference highly significantly different from zero ($p < 0.001$)

e. Difference significantly different from zero ($p < 0.05$)

TABLE 32

Treatment	No. of Animals	Initial Body Weight (g)	Final Body Weight(g)	Total Renal Weight (g)	Renal Wt(mg) / Final Body Wt (g)
Control Diet 15% Casein	6	152±6.82[a]	170±15.00 ↑ b	1.154±0.140 ↑ c	6.763±0.470 ↑ d
Diet supplemented with arginine-free base	6	149±6.62	155±12.50 ↓ b	1.229±0.097 ↓ c	7.930±0.295 ↓ d

The effect of a diet containing 18.5% L-arginine-free base on body weight, kidney and liver weights.

a. Values are means for the groups ± S.D.

b. The difference is not significantly different from zero

c. The difference is not significantly different from zero

d. The difference is highly significantly different from zero ($p < 0.001$)

3.3.5 The Effect of Citrate and Acetate Salts on Renal Weight and Composition

It is obvious from the preceding section that acid produces an increase in renal weight. The effects of an alkalinizing agent were investigated by administering diets containing potassium citrate. In the first experiment in the series, potassium citrate was added to a control diet and to a diet containing 8% glycine. This was done as an additional part of the experiment described in Section 3.3.4 and Tables 27-29, and so can be compared directly with that experiment. The compositions of the diets are shown in Table 8. In the second experiment in the series (performed while the kidneys from the first experiment were being analysed), the effects of sodium citrate were studied as well as those of potassium citrate, and in addition the effects of supplementation of the control diet with sodium and potassium acetate were noted. The compositions of the acetate diets are given in Table 8a.

The results of the first experiment (with potassium citrate) are shown in Table 33 and Figs. 6-8. From these results it is clear that glycine causes a rise in total renal weight which is definitely significant, as was previously described in Section 3,3,3. The addition of potassium citrate alone to the diet gives an increase in total renal weight of 12% which is probably significant ($p < 0.05$). Consideration of the ratio $\frac{\text{renal weight}}{\text{body weight}}$ (Fig. 7) shows that potassium citrate causes a relative increase in renal weight of 15%

which is definitely significant ($p < 0.01$). Again the ratio $\frac{\text{liver weight}}{\text{kidney weight}}$ shows a fall of 10% which is significant in the case of the potassium citrate diet ($p < 0.05$) but shows no significant difference in the case of the glycine diet when the decrease is of the order of 8%. When potassium citrate and glycine are given together, the increase in renal weight is 27%, greater than when either is fed singly, and the increase in $\frac{\text{renal weight}}{\text{body weight}}$ ratio is 30%; the fall in $\frac{\text{liver weight}}{\text{kidney weight}}$ ratio is 22%. A fuller evaluation of these findings requires a consideration of the results of analyses of the kidneys of the animals in this experiment. These are shown in Tables 34-37 and Figs. 9-13.

The following conclusions can be drawn :

1. The addition of potassium citrate to the diet produces a 13% reduction in total renal RNAP ($p < 0.05$). The addition of glycine to the diet produces no significant increase in total renal RNAP as evaluated by student t testing, but Snedecor's F test reveals a 6% increase in left kidney RNAP ($p < 0.05$). In addition, the effect of potassium citrate in producing this reduction in RNAP is greater in the presence of glycine than in its absence, i.e. there is an interaction between glycine and potassium citrate ($p < 0.01$). (See Tables 34 and 37 : Fig. 9).

2./

2. The addition of potassium citrate to the diet produces a 20% reduction in mean RNAP/100 mg ($p < 0.01$). Addition of glycine produces no significant alteration in RNAP/100 mg. When glycine and potassium citrate are both present, there is a 15% fall in RNA/100 mg compared to controls ($p < 0.05$), i.e. there is an interaction between glycine and potassium citrate (see Fig. 10).

3. In the case of the mean $\frac{\text{RNAP}}{\text{DNA}}$ ratio, glycine produces no significant alteration nor does potassium citrate; however, there is a significant increase in the ratio in those animals fed glycine + potassium citrate ($p < 0.05$ with respect to controls and $p < 0.01$ with respect to those animals fed potassium citrate). Therefore, again there is an interaction between glycine and potassium citrate (see Fig. 11).

4. There is a significant increase in $\frac{\text{total RNAP}}{\text{final body weight}}$ in these animals fed glycine + potassium citrate compared to those fed potassium citrate ($p < 0.05$). (See Fig. 12).

5. There is a significant decrease in DNA/100 mg in the group fed glycine, compared to the control group ($p < 0.01$). There is a significant decrease in DNA/100 mg in the group fed glycine + potassium citrate compared to the group fed potassium citrate ($p < 0.01$) and a highly significant decrease compared to the control group ($p < 0.001$). Again there is an interaction between glycine and potassium citrate.

The results of the second experiment in the series are shown in Table 38 and Figs. 14/15. The following conclusions can be drawn :

1. The addition of sodium citrate to the diet causes no significant increase in total renal weight. However, there is an interaction between sodium citrate and glycine (Fig. 14).

2. Again potassium citrate causes a significant increase in total renal weight ($p < 0.05$) and a greater increase in $\frac{\text{total renal weight}}{\text{final body weight}}$ ratio ($p < 0.001$). Also there is an interaction between glycine and citrate.

3. Sodium acetate causes a significant increase in total renal weight ($p < 0.01$). It does not affect the body weight.

4. Potassium acetate causes a fall in body weight ($p < 0.05$). It causes no significant increase in total renal weight but causes an increase in the ratio $\frac{\text{total renal weight}}{\text{final body weight}}$ ($p < 0.01$).

The reasons for these changes are not clear. There appears to be, in the case of potassium citrate, an increase in kidney weight, a slight fall in body weight, coupled with a slight degree of atrophy of the kidney. This is most evident in the presence of glycine. This atrophy affects only RNA, but there is sufficient evidence to encourage a more prolonged study of the effect of DNA. The interaction which occurs between glycine and potassium citrate in altering kidney nucleic acid content suggests that perhaps, when they are administered in combination, some other factor comes into play to reduce kidney RNA and, more especially, kidney DNA. It

seems possible that, if the kidney cells are grossly overhydrated and have a very high intracellular potassium level, this might affect the rate of nucleic acid synthesis. It would be interesting to determine the effect of sodium citrate on kidney composition. Sodium citrate has roughly the same type of effect on kidney weight as potassium citrate, but does not produce a decrease in body weight. Potassium acetate has a similar effect on kidney weight, but produces a marked reduction in body weight. Sodium acetate has a similar effect on kidney weight but no effect on body weight.

The mechanism of the effect is not clear. The fall in RNA might be due to the alkalinizing effect of potassium citrate. Since there is no alteration in the protein content of the kidneys, the effect would appear to be on an early stage of protein synthesis, i.e. by inhibiting the formation of species of RNA. This is, of course, assuming that the effect is due to the alkalinizing properties of potassium citrate rendering superfluous the kidneys' function in the excretion of acid. It could, of course, act via the creation of a state of ionic imbalance (the citrate anion being metabolized, leaving 3 free potassium ions) though this seems less likely. It would be necessary to perform analyses on the kidneys from animals fed sodium citrate and sodium and potassium acetate in order to obtain a better idea of what is occurring.

The increase in renal weight seems most likely to be due to an increase in water content of the kidneys. It was thought that it

might be due to lipid accumulation as the kidneys from the animals fed potassium citrate appeared rather paler than normal. Consequently the left kidneys from the animals in the second experiment were analysed for lipid content. The results are shown in Table 39.

It is clear from these figures that the administration of potassium citrate produces no significant increase in kidney lipid content; the kidneys from the group fed glycine and potassium citrate were not, however, analysed and it would be necessary to perform this set of analyses in view of the fact that there is an interaction between glycine and potassium citrate - the ratio of $\frac{\text{lipid}}{\text{final body weight}}$ while not statistically significant is higher in the group fed 10% potassium citrate.

TABLE 33

Treatment	No. of Animals	Mean Initial Body Weight (g)	Final Body Weight (g)	Total Renal Weight (g)	Renal Weight / Final Body Weight	Liver Wt (g)	Liver Weight / Final Body Weight	Liver Weight / Kidney Wt
Control Diet 15% Casein	5	164	180 ± 3.1	1.213 ± 0.123[ab]	6.73 ± 0.56[c]	8.290 ± 0.289	46.100 ± 3.680	6.890 ± 0.714[d]
Diet containing 8% Glycine	6	160	180 ± 3.6	1.383 ± 0.066	7.69 ± 0.49	9.456 ± 1.183	47.790 ± 5.140	6.220 ± 0.686
Diet containing 10% K. Citrate	6	159	174 ± 2.1	1.343 ± 0.072	7.75 ± 0.65	9.124 ± 0.486	47.630 ± 3.080	6.100 ± 0.419
Diet containing 8% Glycine + 10% K. Citrate	6	158	175 ± 2.9	1.535 ± 0.153	8.70 ± 1.04	9.210 ± 0.828	46.860 ± 3.430	5.380 ± 0.720

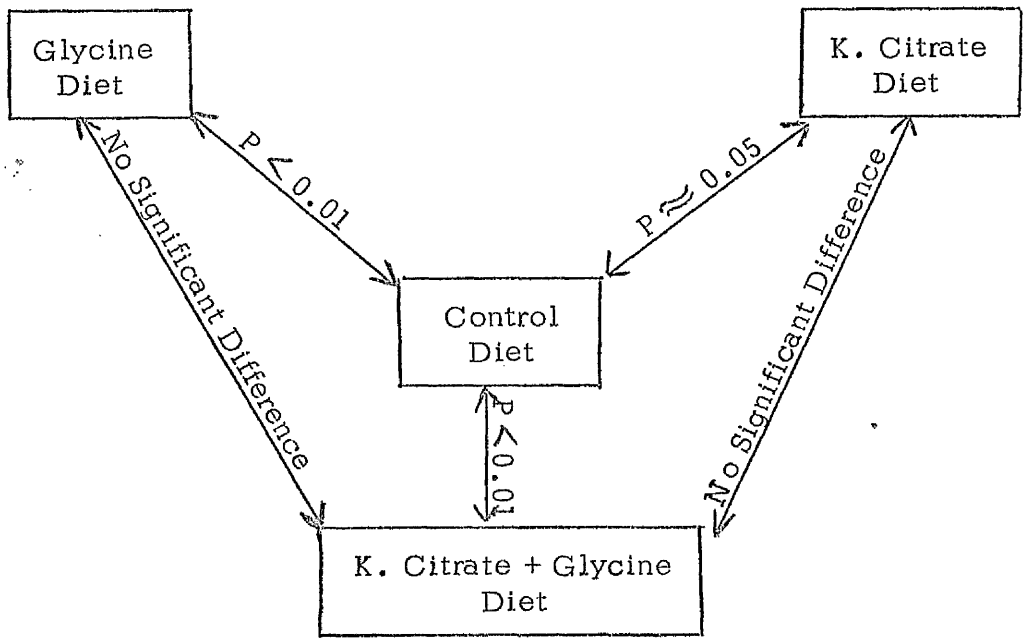
The effect of diets supplemented with 10% potassium citrate and 10% potassium citrate plus 8% glycine compared to control and glycine supplemented diets on body weight and kidney and liver weights.

a. Values are means for the group ± S.D.

b, c, d. Statistical evaluation is shown in Fig. 6, Fig. 7 and Fig. 8 respectively

FIG. 6

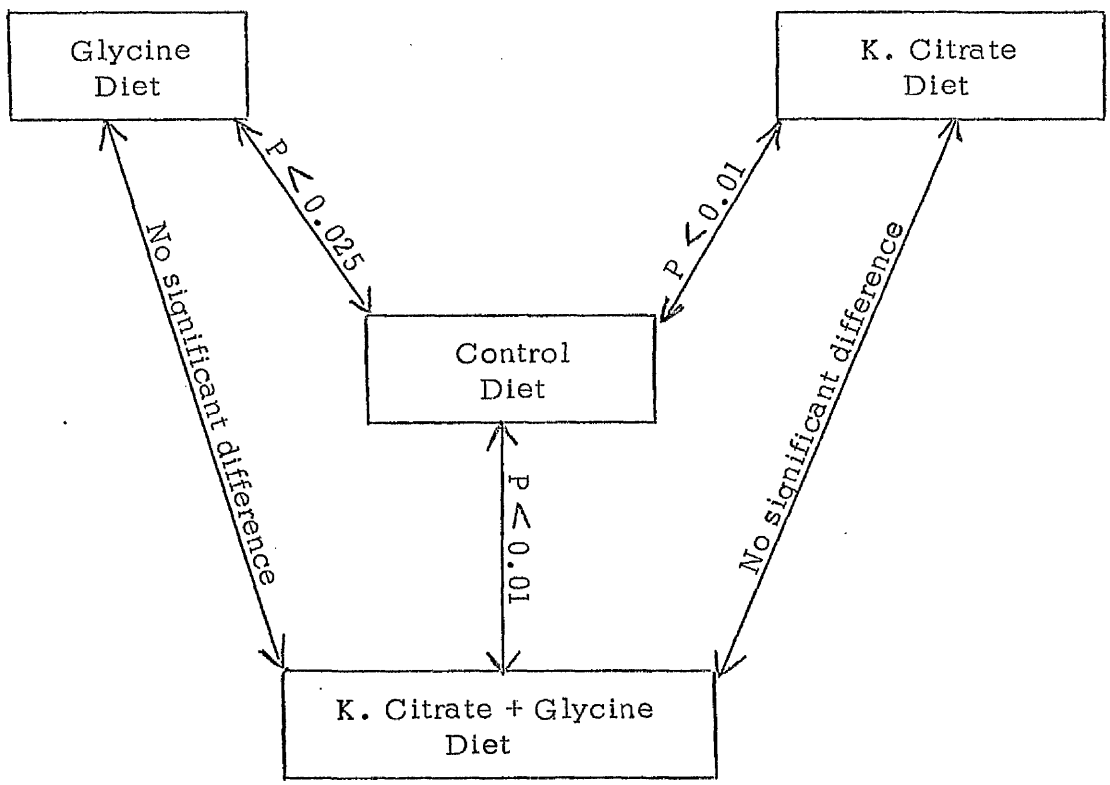
TOTAL RENAL WEIGHT



Statistical evaluation of the differences in total renal weight between the groups shown in Table 33.

FIG. 7

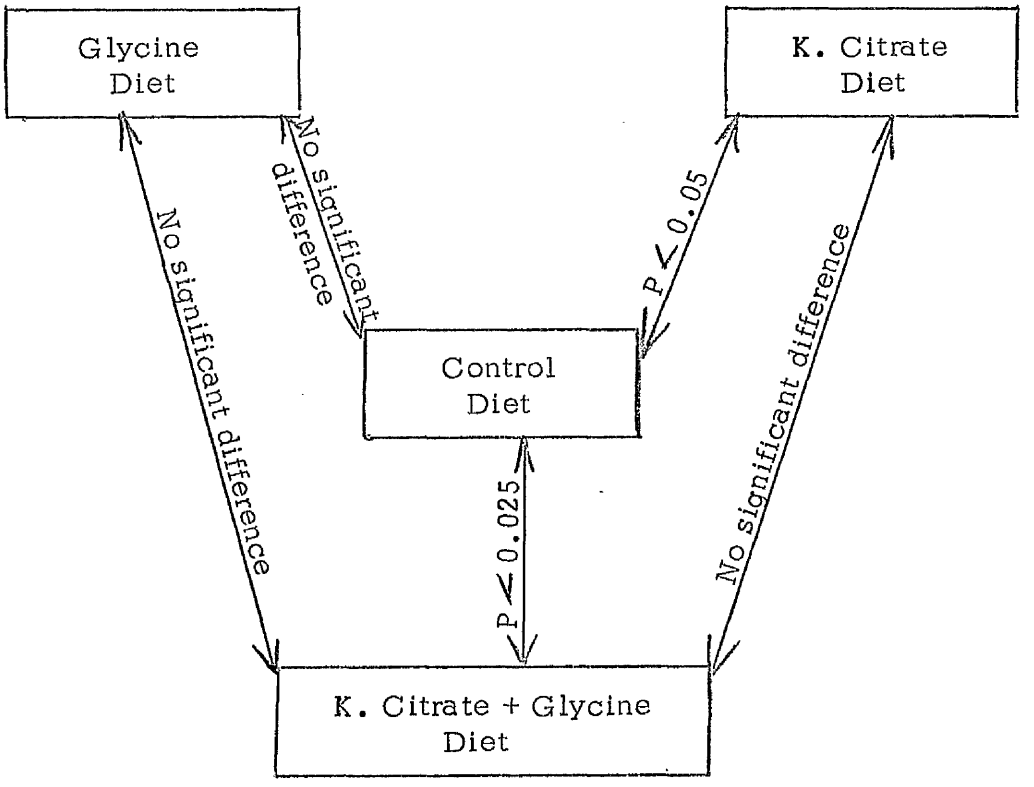
RENAL WEIGHT
BODY WEIGHT



Statistical evaluation of the differences in $\frac{\text{renal weight}}{\text{body weight}}$ ratio between the groups shown in Table 33.

FIG. 8

$$\frac{\text{LIVER WEIGHT}}{\text{KIDNEY WEIGHT}}$$



Statistical evaluation of the differences in $\frac{\text{liver}}{\text{kidney}}$ ratio between the groups shown in Table 33.

TABLE 34

Treatment	No. of Animals	RNAP/Kidney (μg)		RNAP/100 mg (μg)		Mean RNAP/DNA	Total Kidney RNAP (μg) / Final Body Wt (g)
		Left	Right	Left	Right		
Control Diet (15% Casein)	5	233 ± 19.6[ab]	240 ± 24.8	39.4 ± 1.2[c]	38.6 ± 0.4	0.0909 + 0.0100[d]	2.73 ± 0.37[e]
Diet containing 8% Glycine	6	252 ± 42.0	251 ± 40.5	37.2 ± 5.9	36.0 ± 6.3	0.1039 ± 0.0203	2.82 ± 0.44
Diet containing 10% K. Citrate	6	199 ± 24.6	218 ± 21.5	30.8 ± 4.8	31.4 ± 4.0	0.0833 ± 0.0125	2.40 ± 0.30
Diet containing 8% Glycine + 10% K. Citrate	6	236 ± 33.7	269 ± 25.8	31.5 ± 4.6	34.7 ± 5.4	0.1131 ± 0.0217	2.90 ± 0.37

The effects of supplementation of a control diet and a diet containing 8% glycine with 10% potassium citrate on kidney RNAP content and RNAP/DNA ratio.

a. Values are means for the groups ± S.D.

b. Statistical evaluation is shown in Fig. 9, Table 37
c. Statistical evaluation is shown in Fig. 10
d. Statistical evaluation is shown in Fig. 11
e. Statistical evaluation is shown in Fig. 12

TABLE 35

Treatment	No. of Animals	Protein/Kidney mg (µg)		Protein/100 mg (µg)	
		Left	Right	Left	Right
Control Diet (15% Glycine)	5	112 ± 20[a]	122 ± 19	18.9 ± 2.2	19.7 ± 2.4
Diet containing 8% Glycine	6	126 ± 10	116 ± 12	18.3 ± 1.3	16.6 ± 1.7
Diet containing 10% K. Citrate	6	114 ± 7	130 ± 22	17.4 ± 1.2	18.7 ± 3.7
Diet containing 8% Glycine + 10% K. Citrate	6	124 ± 9	130 ± 10	16.6 ± 0.9	16.5 ± 0.8

The effects of supplementation of a control diet and a diet containing 8% glycine with 10% potassium citrate on kidney protein content.

a. Values are means for the groups ± S.D.

TABLE 36

Treatment	No. of Animals	DNA/KIDNEY (µg)		DNA/ 100 mg (mean) (µg)	Total DNA (µg) / Final Body Wt (g)
		Left	Right		
Control Diet (15% Glycine)	5	2676 ± 583[a]	2597 ± 458	[b] 436 ± 48	29.34 ± 3.67
Diet containing 8% Glycine	6	2564 ± 190	2242 ± 188	346 ± 28	27.00 ± 3.11
Diet containing 10% K. Citrate	6	2384 ± 410	2754 ± 306	381 ± 53	30.00 ± 3.85
Diet containing 8% Glycine + 10% K. Citrate	6	2202 ± 286	2333 ± 282	296 ± 33	26.08 ± 3.86

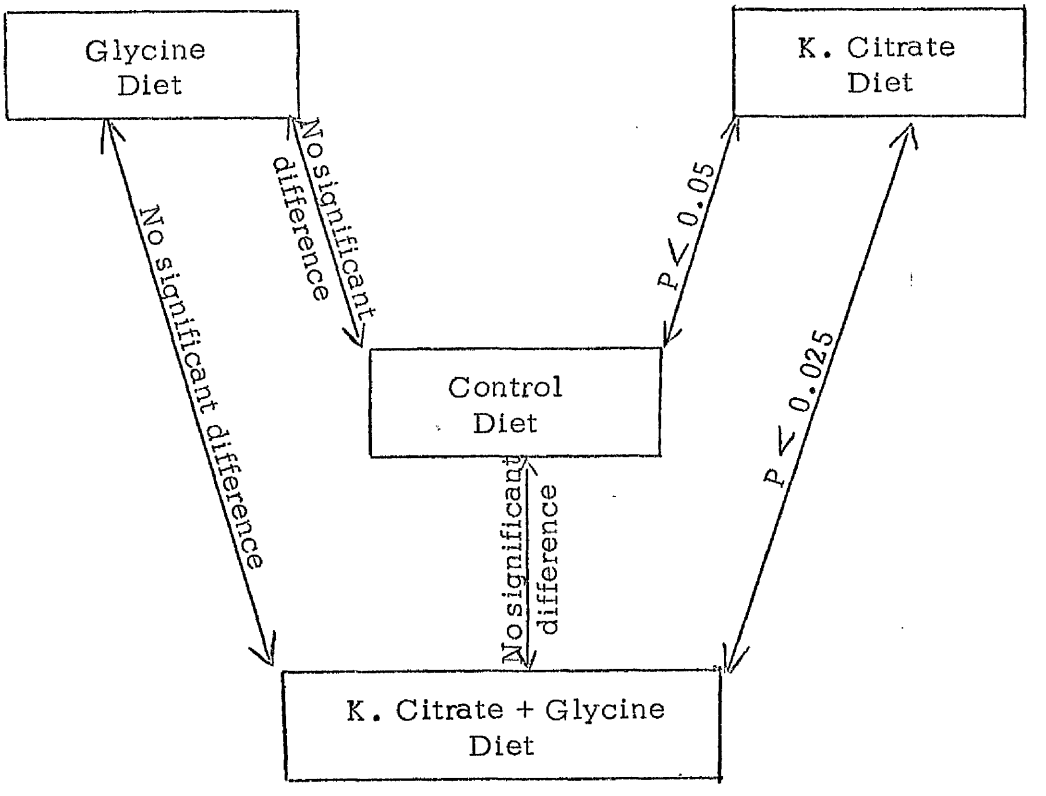
The effects of supplementation of a control diet and a diet containing glycine with 10% potassium citrate on kidney DNA content.

a. Values are means for the groups ± S.D.

b. Statistical evaluation is shown in Fig. 13

FIG. 9

TOTAL KIDNEY RNAP



Statistical evaluation of the differences in total kidney RNAP (left and right kidneys) between the groups shown in Table 34 (statistics performed by student t testing).

TABLE 37

LEFT KIDNEY RNAP

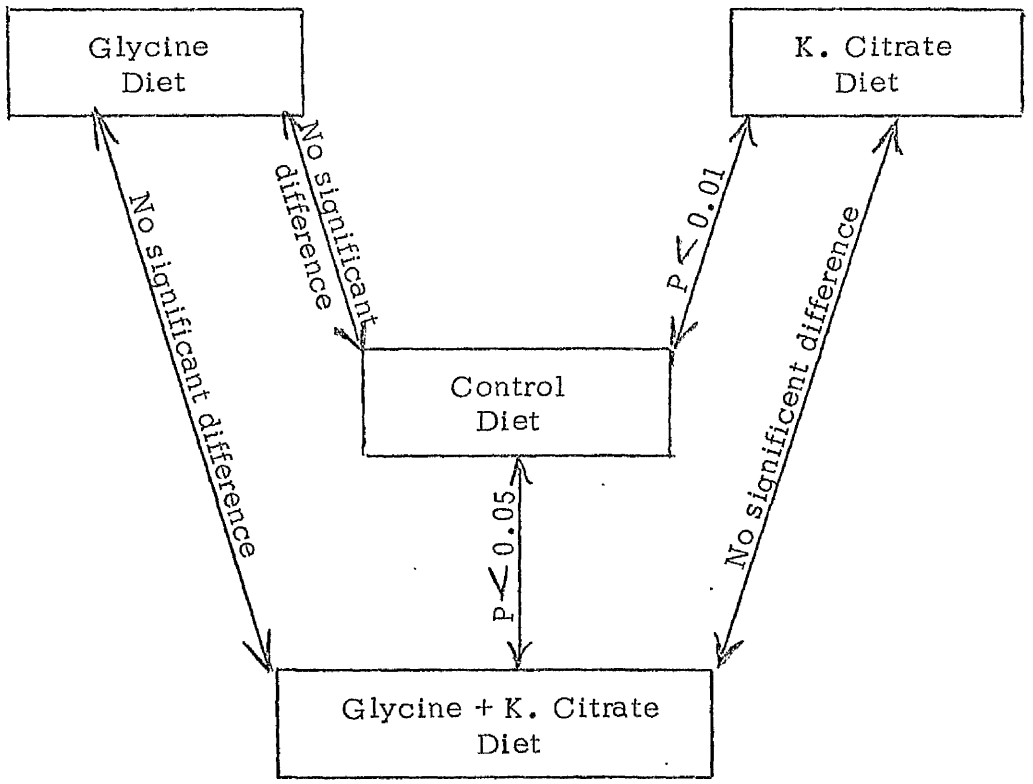
Analysis of variance in left kidney RNAP between the groups shown in Table 34. Performed by Snedecor's F test (Moroney, 1951).

Variation due to :

Glycine	Significant	$P < 0.05$ and almost ≈ 0.01
Potassium Citrate	"	$P < 0.05$
First Order Interaction	"	$P < 0.01$

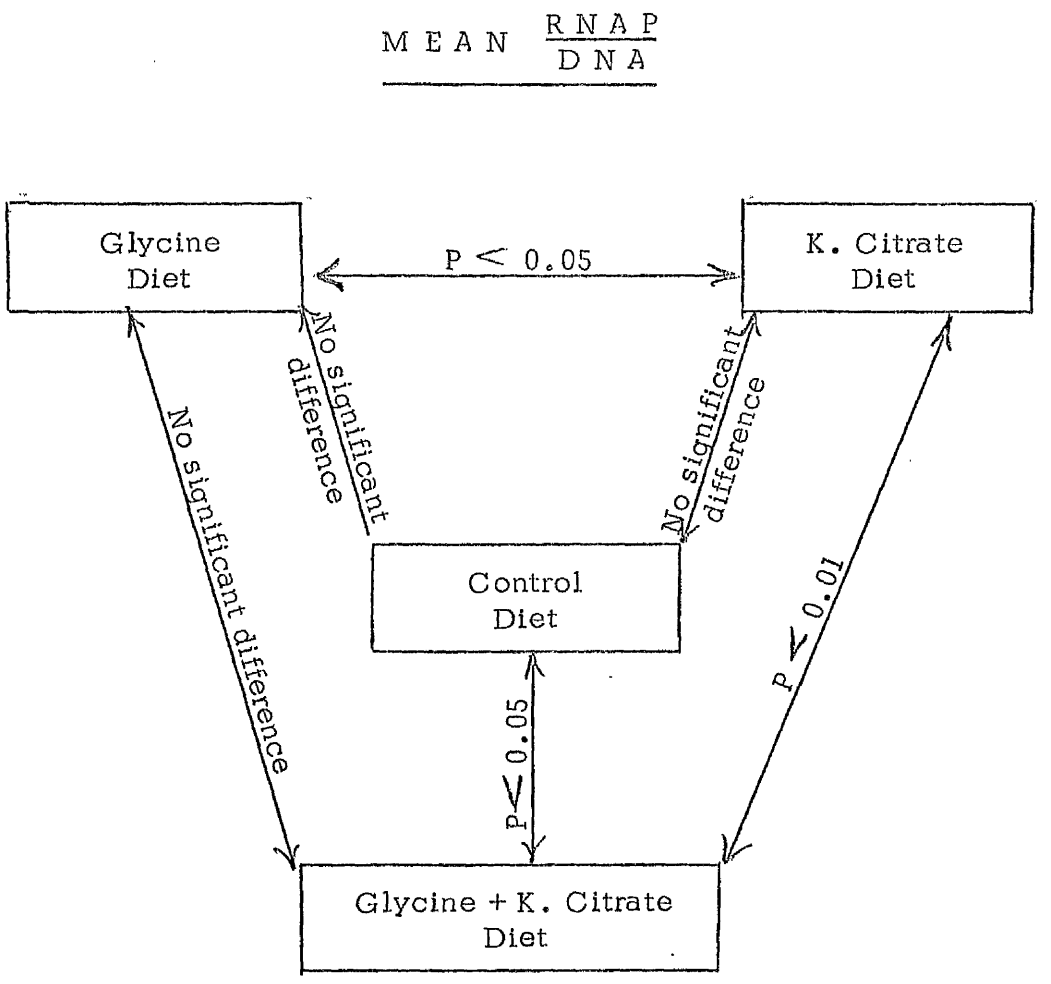
FIG. 10

MEAN RNAP/100 mg



Statistical evaluation of the differences in mean RNAP/100 mg between the groups shown in Table 34. Analysis performed by student t test.

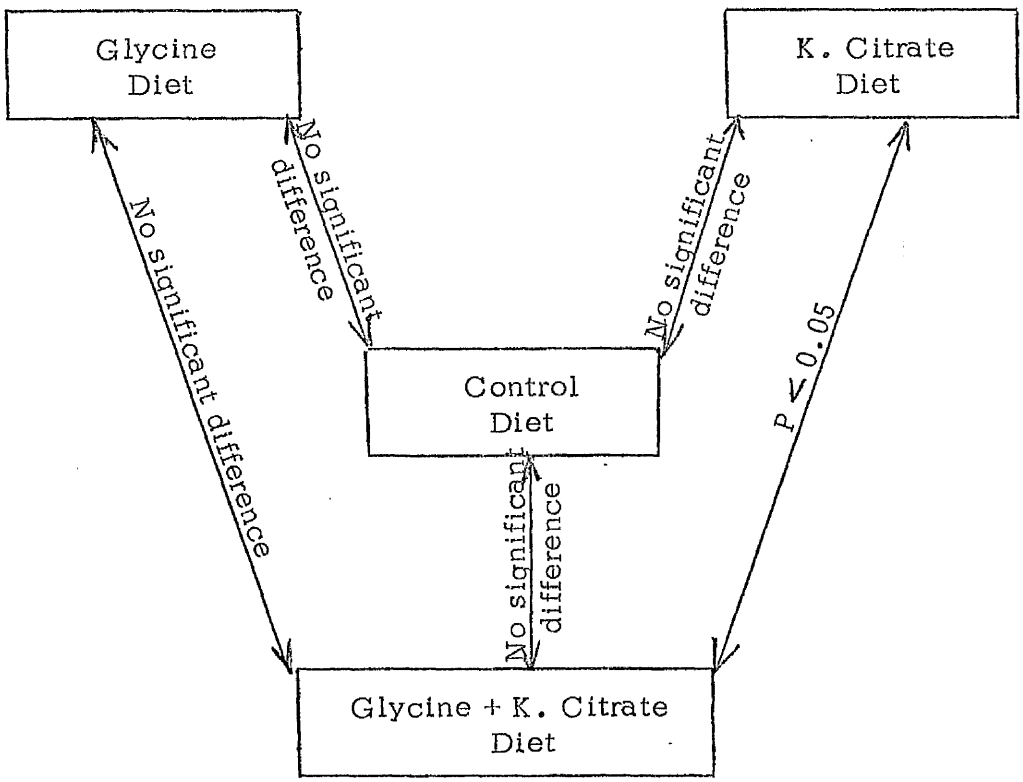
FIG. 11



Statistical analysis of the differences in $\frac{RNAP}{DNA}$ ratios between the groups shown in Table 34. The analysis was performed by Student t testing.

Fig. 12

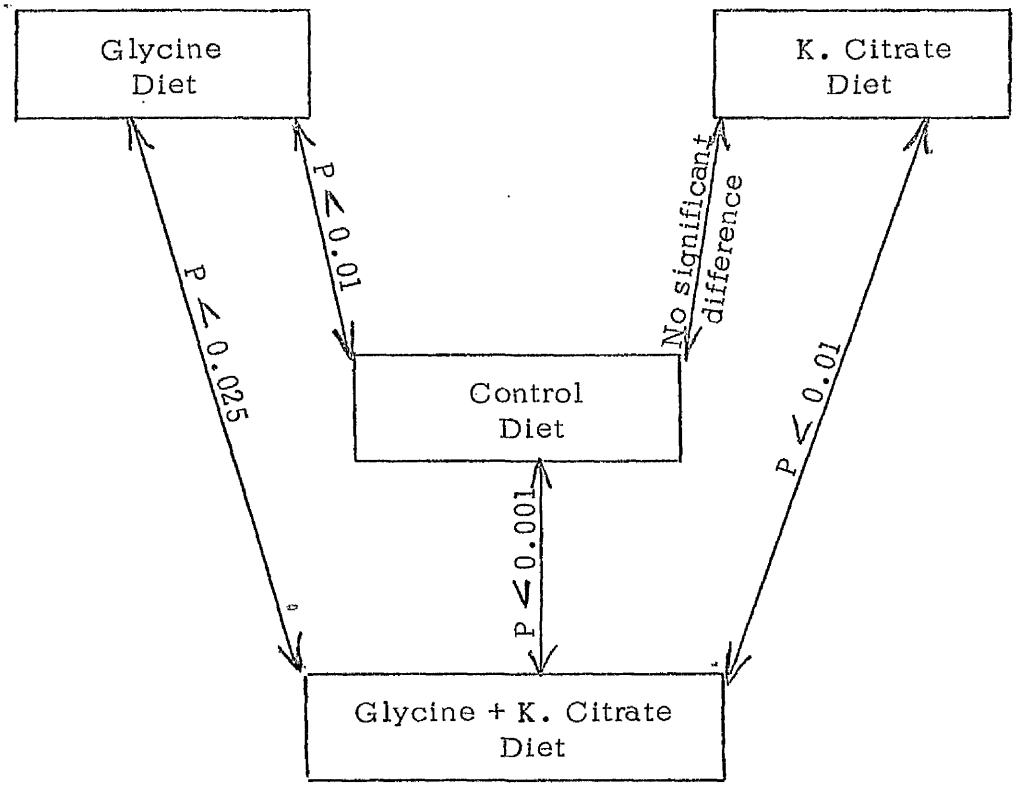
TOTAL KIDNEY RNAP
FINAL BODY WEIGHT



Statistical analysis of the differences in $\frac{\text{total kidney RNAP}}{\text{final body weight}}$ between the groups shown in Table 34.

FIG. 13

MEAN DNA/100 mg



Statistical analysis of the differences in mean DNA/100 mg between the groups shown in Table 36.

TABLE 38

Treatment	No. of Animals	Mean Weight Gain	Final Wt	Total Renal Wt (g)	Total Renal Wt(mg) / Final Body Wt (g)
Control Diet (15% glycine)	5	20	160±15.2[a]	1.091±0.120	6.820±0.387
Diet containing 8% glycine	5	18		1.285±0.066	8.620±1.30
Diet containing 9% Na Citrate	5	27		1.144±0.044	7.420±0.713
Diet containing 10% K. Citrate	5	31		1.226±0.118	8.440±0.270
Diet containing 8% glycine + 9% Na Citrate	5	21		1.357±0.080	8.920±0.610
Diet containing 8% glycine + 10% K. Citrate	5	12		1.317±0.140	8.800±0.600
Diet containing 14% Na Acetate	5	18		1.327±0.104	8.680±0.878
Diet containing 10% K. Acetate	5	-3	136±11.4	1.184±0.111	8.76 ±1.110

b

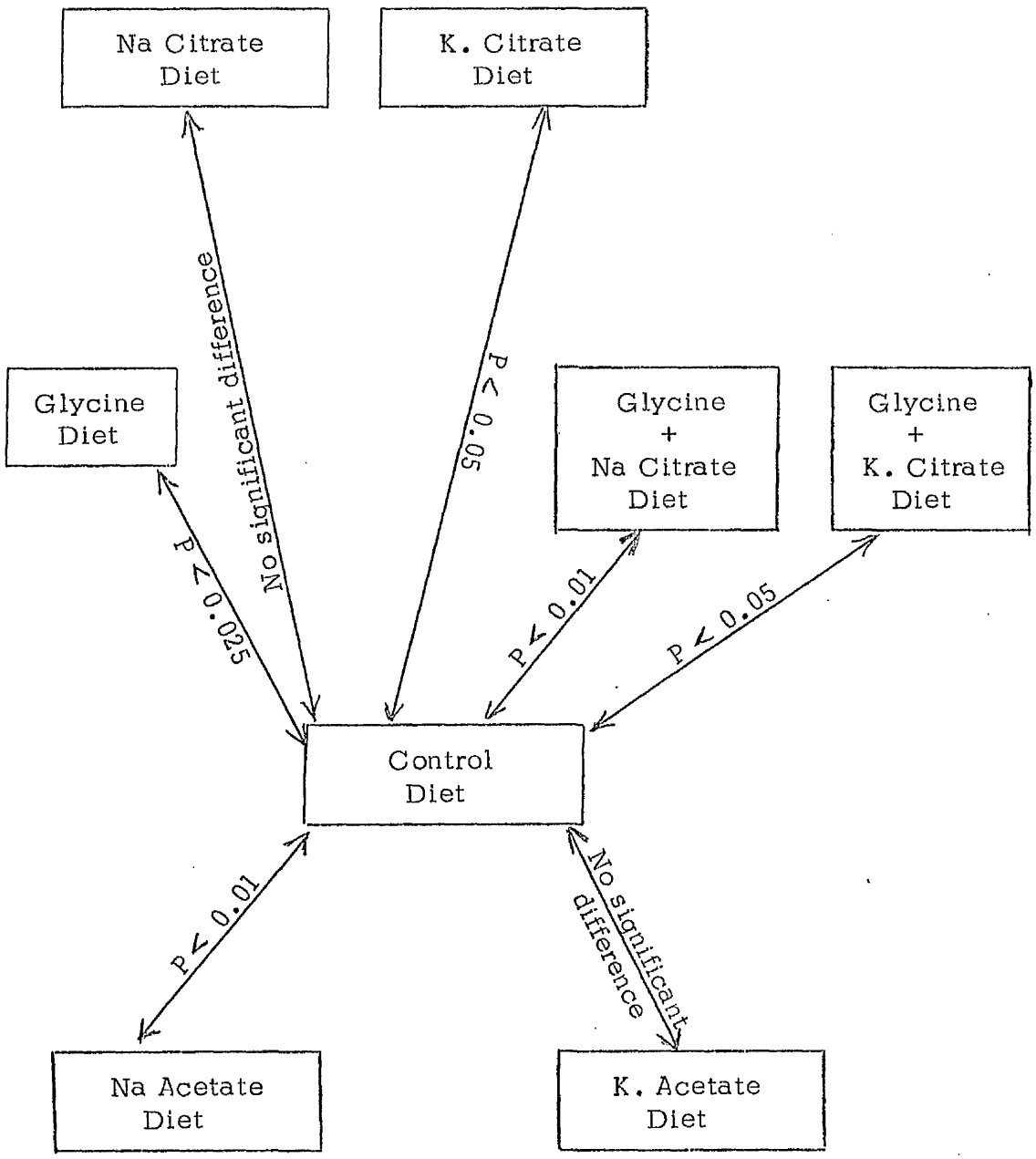
The effects of diets containing sodium and potassium citrate, glycine and sodium and potassium acetate on body weight and kidney weight.

a. Values are means for the groups ± S.D.

b. Difference significantly different from zero ($p < 0.05$)

FIG. 14

TOTAL RENAL WEIGHT



Statistical evaluation of the differences in total renal weight between the groups shown in Table 38.

FIG. 15

TOTAL RENAL WEIGHT
FINAL BODY WEIGHT

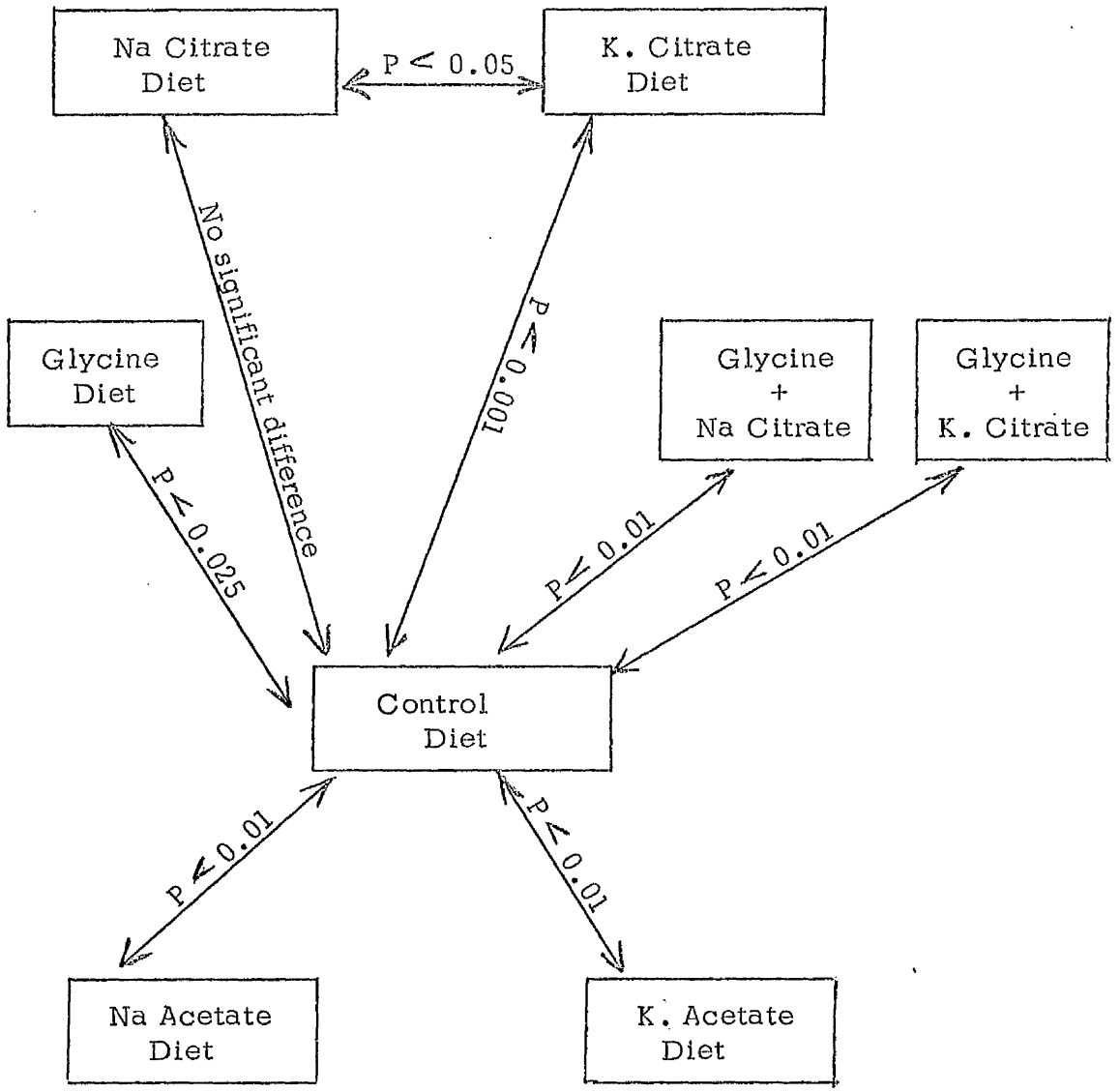


TABLE 39

Treatment	No. of Animals	Left Kidney Wt (g)	Left Kidney Lipid (mg)	Lipid/100 mg (mg)	Lipid mg / Final Body Wt (g)
Control Diet (15% Casein)	5	0.532±0.062[a]	21.30±1.50	4.06 ± 0.67	0.134±0.023
Diet containing 10% K.Citrate	5	0.614±0.080	22.80±2.89	3.76 ± 0.45	0.157±0.013

The effect of a diet containing 10% potassium citrate on kidney lipid levels.

a. Values are means for the groups ± S.D.

3.3.6 The Effect of Attempted Increase in Glucuneogenesis of Body Weight, Liver and Kidney Weights

The kidney, like the liver, possesses all the enzymes of gluconeogenesis and plays a major role in the process. An attempt was made by dietary means to increase the gluconeogenic activity of the kidney to determine whether this would cause a measurable increase in renal weight. This was done by administering a high fat diet to one group of animals and a control diet to another group. The experiment lasted 6 days and the animals were weighed and sacrificed as before. The results are shown in Table 40, and the compositions of the diets are given in Table 9. It can be seen that there was no difference in kidney or liver weights.

A further experiment was undertaken in which one group of animals was fed a protein-free high fat diet for 6 days while the control group received a protein-free high carbohydrate diet for the same length of time. This might reasonably be expected to exaggerate any latent response to the diets used in the first experiment. The results of this experiment are shown in Table 41 and the composition of the diets is given in Table 9. There is no difference in total renal weight. The mean liver weight of the group fed the high fat protein-free diet is higher than the mean liver weight of the group fed the high carbohydrate-protein-free diet; however, P is just greater than 0.05, but the ratio $\frac{\text{liver weight}}{\text{final body weight}}$ is significantly greater in the former group ($p < 0.01$) as is also the ratio $\frac{\text{liver weight}}{\text{kidney weight}}$ ($p < 0.05$). This suggests that the liver weight is increasing relative to body weight, and that the high fat protein-free diet is affecting the liver, though not the kidney. The result might be due to hypertrophy of the liver or simply to accumulation of lipid.

TABLE 40

Treatment	No. of Animals	Mean Weight Gain(g)	Total Renal Weight (g)	Liver Weight (g)
Control Diet (11.2% fat)	6	17	1.144 ± 0.110[a]	6.668 ± 0.280
High Fat Diet (30% fat)	6	20	1.198 ± 0.052	6.672 ± 0.260

The effect of administration of a high fat diet compared to a control diet on kidney and liver weight.

a. Values are means for the groups ± S.D.

TABLE 41

Weights	Treatment	
	Protein-Free Diet	Protein-Free High Fat Diet
No. of Animals	6	6
Initial Body Weight (g)	171.00 ± 5.83[a]	169.00 ± 7.35
Final Body Weight (g)	155.00 ± 12.1	149.00 ± 5.74
Total Renal Weight (g)	0.99 ± 0.061	1.00 ± 0.128
Liver Weight (g)	6.08 ± 0.55 [b]	6.93 ± 0.77
Renal Weight / Body Weight	6.43 ± 0.4	6.70 ± 0.66
Liver Weight / Body Weight	39.43 ± 3.0 [c]	46.38 ± 4.16
Liver / Kidney	6.16 ± 0.58 [d]	6.49 ± 0.44

The effect of administration of a high fat-protein-free diet compared to a high carbohydrate-protein-free diet on body weight, kidney and liver weights.

a. Values are means for the groups ± S.D.
b. Difference not significantly different from zero ($p > 0.05$)
c. Difference significantly different from zero ($p < 0.01$)
d. Difference significantly different from zero ($p < 0.05$)

3.4 THE HUMORAL THEORY

Numerous experiments have been performed to determine whether growth in the renoprival state is stimulated by a humoral agent (see Section 1). These have given contradictory results. In part, this has been due to the fact that the investigators have used as their index of growth a change in the mitotic index or the incorporation of radio-active isotope into DNA. Halliburton (1966) and others have shown that increase in DNA plays a small role in the process; therefore, in the present study a more sensitive index of hypertrophy was sought. It was decided to use the incorporation of isotope into RNA and protein as well as DNA and to seek a system which allowed the detection of a small quantity of any humoral agent which might be present in the blood of nephrectomized animals.

Dickson ~~Dixon~~ and Leslie (1965, ~~1966~~), Dickson ~~Dixon~~ (1966) describe an in vitro system of adult guinea pig kidney cells which remain viable and aggregate on micropore filters. The cells continue to metabolize but have a very low rate of cell division. The disaggregated single cell suspension begins to form primitive tubular structures 4 days after setting up the cultures; these structures become more organized and are well formed tubules at 8-10 days. This system, by allowing the use of small numbers of kidney cells would permit the detection of a small quantity of stimulant factor. The technique was attempted using young adult rat kidney cells, and is described in Section 2.5.

Growth was estimated using isotope incorporation. In the earlier experiments, designed to test the system, ^{14}C leucine was used to estimate protein synthesis and RNA synthesis; leucine enters mainly into protein synthesis but also enters RNA synthesis. Tritiated (^{3}H) cytosine was used to estimate RNA and DNA synthesis. In later experiments, designed to test for the presence of plasma factors, ^{3}H leucine and ^{14}C thymidine, which enters only DNA synthesis, were used to estimate growth.

Trial experiments were performed on several occasions. Generally 5-6 hours were required for setting up the cultures. In these trial experiments, some of the cultures were removed for histological purposes while others were tested for isotope incorporation. Histological preparation proved to be the most difficult part of the technique. The method described by ~~Dixon~~ DICKSON (1966) proved to be too traumatic for the small number of cells used in these experiments and finally the somewhat simplified schedule given in Section 2.6 was adopted.

3.4.1 In Vitro Experiment No. 1 : To Test the System

An initial preparatory experiment was undertaken in order to test the system and determine roughly the isotope incorporation which might be expected at various times after initial incubation of the cultures. Consequently an experiment was set up with 12 filter

wells, each containing 5×10^6 cells. Following 6-hour incorporations with 5 μC ^{14}C leucine and 5 μC ^{3}H cytosine duplicate cultures were harvested at 24 hours, 36 hours, 48 hours, 3 days, 6 days and 8 days. The results are shown in Table 42. In filter well No. 2 of the cultures harvested at 24 hours, the low activity was due to ill-fitting apparatus causing excessive alkalization due to loss of CO_2. This can therefore be excluded from the calculations. It is clear, however, that, while there is a gradual loss of activity over 8 days, the cells remain viable and continue to synthesize protein and nucleic acid for the first 3-4 days. In later experiments the isotopes used were ^{3}H leucine and ^{14}C thymidine in order to obtain an estimate of DNA synthesis as well as protein and nucleic acid.

There is a fairly wide scatter in isotope return from duplicate cultures and it is obvious that to obtain a definite result there would need to be a large difference between cultures incubated with test and control rat plasmas. From the results obtained in this initial preparatory experiment, it was decided to harvest all cultures at 3 days in the experiments designed to test the effect of plasma. At this time there is fairly high activity in the cultures and at the same time the cells begin to form primitive tubular structures (~~Dixon,~~ Dickson 1966).

TABLE 42

Time of Harvesting (days)	Counts/Min/10^6 Cells ^{14}C Leucine[ac]	Counts/Min/10^6 Cells ^{3}H Cytosine[bd]
1	362	100
1.5	386	70
2	100	18
3	223	41
6	5	1
8	5	0.4

Isotope recovery from cultures harvested at various times.

a. Values are the means of 2 cultures.

b. Values are the means of 2 cultures.

c. Values represent the isotope recovery after a 6-hour incorporation of 5 μ C of ^{14}C leucine.

d. Values represent the isotope recovery after a 6-hour incorporation of 5 μ C of ^{3}H cytosine.

3.4.2 Incubation with Sham-Operated and Nephrectomized Rat Plasma

It was the opinion of the investigator that the result of the foregoing experiment showed that aggregating adult kidney cell cultures formed a biochemically active colony which could be used to test for the presence of a stimulatory factor in the blood of nephrectomized rats. Three such experiments were undertaken and were performed as already described except that all cultures were harvested at 3 days. There were 8 cultures in each experiment, 4 test and 4 control. One hour before isotope incorporation was begun, the medium was changed from one containing 5% calf serum to one containing 15% nephrectomized or 15% sham-operated rat plasma. The plasma was withdrawn from animals nephrectomized 36 hours previously; the animals were male rats weighing 120 g, i.e. the same sex and weight as the animal from which the cultured tissue was obtained. Isotope was incorporated for 6 hours; 5 μc ^{3}H leucine was used to estimate protein and to a lesser extent RNA synthesis and 5-10 μC ^{14}C thymidine was used to estimate DNA synthesis.

3.4.2.1 The Effect of 15% Rat Plasma on 30×10^6 Cells

In the first experiment in the series the effect of 15% sham-operated and nephrectomized rat plasma was studied, using 30×10^6 cells per filter well. The results are shown in Table 43. It is seen from these results that the specific activity of ^3H leucine in the group incubated with plasma from nephrectomized animals is approximately twice that of the group incubated with plasma from nephrectomized animals. This suggests that a stimulant factor is present in the blood of nephrectomized animals, However, there is a wide scatter between cultures within the same group and statistical evaluation did not support the theory. Therefore, it was decided to repeat the experiment, this time using a smaller number of cells per culture vessel and the same concentration of rat plasma. It can also be seen that there is no difference in incorporation of ^{14}C thymidine and that this incorporation is very low. This suggests that any stimulant factor which is present acts by causing hypertrophy, i.e. RNA and protein synthesis and not on DNA synthesis. This is in keeping with the situation obtaining in the whole animal (Halliburton, 1966).

TABLE 43

Treatment	No. of Cells per Culture	Specific Activity of ^{3}H Leucine Recovered[a] (d.p.m/10^6 cells)	Specific Activity of ^{14}C Thymidine Recovered[b] (d.p.m/10^6 cells)
Incubated with 15% plasma from right unilaterally nephrectomized rats	30×10^6	1300 ± 780[c]	0.84 ± 0.40 (BACKGROUND)
Incubated with 15% plasma from sham nephrectomized rats	30×10^6	673 ± 205	0.81 ± 0.28 (BACKGROUND)

The effect on 30×10^6 adult rat kidney cells aggregating in vitro of a 6-hour incubation with plasma from unilaterally nephrectomized rats compared to the effect of incubation with plasma from sham-operated animals.

a. After 6-hour incorporation of 5 μC ^{3}H leucine

b. After 6-hour incorporation of 5 μC ^{14}C thymidine

c. Values are means for the groups ± S.E.

3.4.2.2 The Effect of 15% Rat Plasma on 10×10^6 Cells

The results of this experiment are shown in Table 44. In this experiment also the specific activity of ^3H leucine incorporation in the group incubated with plasma from nephrectomized rats is more than twice that in the group incubated with plasma from sham operated rats. In this experiment the total return of isotope was very low. This may in part be due to decay since, because of pile-up at the scintillation counter, several days elapsed between preparation and counting. The specific activity of ^{14}C thymidine recovered was twice as great in the test as the control group. However, the total incorporation of ^{14}C thymidine was very low and it is difficult to draw definite conclusions regarding DNA synthesis from these figures. However, again statistical evaluation of the results does not support the theory.

TABLE 44

Treatment	No. of Cells per Culture	No. of Cultures	Specific Activity of ^{3}H Leucine Recovered[ab] (d.p.m/10^6 cells)	Specific Activity of ^{14}C Thymidine Recovered[c] (d.p.m/10^6 cells)
Incubated with 15% plasma from right unilaterally nephrectomized rats	10×10^6	3	29.1 ± 8.2[d]	3.27 ± 1.35 (Background)
Incubated with 15% plasma from sham nephrectomized rats	10×10^6	4	14.7 ± 3.90	1.25 ± 0.25 (Background)

The effect on 10×10^6 adult rat kidney cells aggregating in vitro of a 6-hour incubation with plasma from unilaterally nephrectomized rats compared to the effect of incubation with plasma from sham operated animals.

a. The isotope return in this case is very low. This was due to a considerable delay in counting due to technical difficulties with the liquid scintillation counter.

b. After 6-hour incorporation of 5 μC ^{3}H leucine

c. After 6-hour incorporation of 5 μC ^{14}C thymidine

d. Values are means for the groups ± S.E.

3.4.2.3 The Effect of 15% Rat Plasma on 3×10^6 Cells

It might be reasonable to suppose that, if the results of the two previous experiments indicate the presence of a humoral stimulant in unilaterally nephrectomized animals, the effect might be seen even more clearly by further reducing the number of cells per filter vessel. Therefore the experiment was repeated using 3×10^6 cells per vessel, and maintaining the concentration of plasma in the medium at 15%. The results of this experiment are shown in Table 45. It can be seen from this table that the mean specific activity of ^3H leucine recovered from the cultures incubated with plasma from nephrectomized animals is 14 times greater than the mean specific activity of ^3H leucine recovered from the control cultures. This might suggest a very greatly increased protein and RNA metabolism. Interestingly, and surprisingly, in view of the findings in the previous experiments, the specific activity of ^{14}C thymidine recovered from the test cultures is 15 times greater than that from the control cultures. This might suggest a greater degree of DNA synthesis. It is interesting that the suggested degree of increase in protein and RNA metabolism is of the same order as that of DNA metabolism, although the total incorporation of ^3H leucine is much greater than the total incorporation of ^{14}C thymidine. It must be remembered that twice as much ^{14}C thymidine was added to each vessel (10 μC instead of 5 μC), but this is very unlikely to have made any difference to the final result.

TABLE 45

Treatment	No. of Cells per Culture	No. of Cultures	Specific Activity of ^{3}H Leucine Recovered[a] (d.p.m/10^6 cells)	Specific Activity of ^{14}C Thymidine Recovered[b] (d.p.m/10^6 cells)
Incubated with 15% plasma from right unilaterally nephrectomized rats	3×10^6	4	17,815 ± 10,600[c]	494 ± 215
Incubated with 15% plasma from sham nephrectomized rats	3×10^6	4	1,290 ± 700	32 ± 30

The effect on 3×10^6 adult rat kidney cells aggregating in vitro of incubation with plasma from unilaterally nephrectomized rats compared to the effect of incubation with plasma from sham operated animals.

a. After 6-hour incorporation of 5 μC ^{3}H leucine

b. After 6-hour incorporation of 10 μC ^{14}C thymidine

c. Values are means for the groups ± S.E.

The interpretation of the results from this last experiment in the series is rather difficult. Student t testing shows that there is not a significant difference in isotope incorporation between the two groups. However, it can be seen from Table 45 that, after allowing for the standard error, there is no overlap between the two groups in this experiment. This, coupled with the fact that in each of the three experiments the group incubated with plasma from nephrectomized rats has a higher incorporation of isotope into protein and RNA, gives ground for the gravest suspicion that a humoral agent is present in the plasma of unilaterally nephrectomized rats.

Assuming this to be the case, it can be seen why previous attempts to discover the presence of such a factor have failed. These have been concerned principally with whole animal cross-circulation experiments. Now, in the present case, a positive result was obtained by exposing 3×10^6 cells to plasma for 6 hours; each filter well contained 0.225 ml of plasma in a total volume of 1.8 ml. A simple calculation shows that the amount of blood which would have to be cross-circulated into a whole animal to bathe 2 kidneys with a similar concentration of plasma for 1-2 days would be enormous, and in fact impossible to achieve.

It would appear that this factor in the plasma produces a hypertrophy at lower relative concentrations and a hyperplasia at higher relative concentrations as DNA metabolism is affected only when there are 3×10^6 cells per culture vessel. Also, the factor need not be a stimulator of growth, but could be an inhibitor which is not present in the plasma of nephrectomized rats. The present investigator believes that a stimulatory factor would seem more likely.

3.5 INVESTIGATION OF METHODS REPUTED TO EXAGGERATE THE RESPONSE TO UNILATERAL NEPHRECTOMY

3.5.1 Partial Hepatectomy

Paschkis, Goddard, Cantarow and Adibe (1959) found that rats subjected to combined unilateral nephrectomy and partial hepatectomy developed kidneys 50% heavier than those in unilaterally nephrectomized controls. Simpson (1961), however, found no alteration in ^{32}P incorporation into kidney DNA in normal rats as a result of partial hepatectomy. Paschkis et al. claimed that the increased response they obtained was due to some general growth promoting mechanism activated by partial hepatectomy; the results of Simpson are at variance with this. It was decided to investigate the response to unilateral nephrectomy in partially hepatectomized animals in order to settle the issue.

Consequently 16 animals were divided into 2 groups of equal weight. To 8 of these a two-thirds partial hepatectomy was performed and the remaining 8 had sham hepatectomies. All the animals were right-unilaterally nephrectomized immediately after the partial hepatectomy or sham hepatectomy. They were caged individually until 96 hours after the operations when they were sacrificed and the kidneys and livers removed and weighed.

The results of this experiment are shown in Tables 46 and 47. It is clear that there is no statistically significant difference in the response to unilateral nephrectomy between the 2 groups. There appears to be a tendency towards a decrease in the response in the group which was partially hepatectomized; however, when the $\frac{\text{left}}{\text{right}}$ ratio is corrected for body weight, it is clear that the response to unilateral nephrectomy is identical in the 2 groups.

In Table 47, the response of the fragment remaining after partial hepatectomy is estimated. The liver recovered 73.5% of its original weight 96 hours after operation. This figure corresponds with other estimates of liver weight recovery at this time. Brues, Drury and Brues (1936) give a figure of 76.7% recovery at 96 hours, when corrected for body weight loss. Therefore it can be concluded that unilateral nephrectomy has no effect on the growth response of the liver fragment remaining after two-thirds partial hepatectomy.

These results disprove the theory that some general growth promoting agent is liberated following partial hepatectomy or unilateral nephrectomy.

TABLE 46

	Sham Hepatectomy followed by right Unilateral Nephrectomy	$\frac{2}{3}$ Partial Hepatectomy followed by right Unilateral Nephrectomy
No. of Animals	8	8
Initial Body Weight	222 ± 4.070[a]	221 ± 3.610
Final Body Weight	210 ± 2.150	199 ± 9.540
Right Kidney Weight	0.721 ± 0.013	0.775 ± 0.054
Left Kidney Weight	0.930 ± 0.080	0.930 ± 0.054
$\frac{\text{Left}}{\text{Right}}$ Ratio	1.310 ± 0.160	1.190 ± 0.110
$\frac{\frac{\text{Left}}{\text{Right}} \text{ Ratio}}{\text{Final Body Weight}}$	0.0062 ± 0.0006	0.0060 ± 0.0008

The effect of $\frac{2}{3}$ partial hepatectomy on the response to unilateral nephrectomy.

a. Values are means for the groups ± S.D.

TABLE 47

	Sham Hepatectomy followed by right Unilateral Nephrectomy	$\frac{2}{3}$ Partial Hepatectomy followed by right Unilateral Nephrectomy
No. of Animals	8	8
Weight of Liver Excised	0	6.11 ± 0.93
Final Liver Weight	9.08 ± 0.73[a]	6.35 ± 0.91
% Liver Weight Recovered		69.94
% Liver Weight Recovered (corrected for Final Body Weight)		73.45 ± 5.11

The effect of right unilateral nephrectomy on the response to $\frac{2}{3}$ partial hepatectomy.

a. Values are means for the groups ± S.D.

3.5.2 Renal Denervation

Allen and Mann (1935) report a higher response to unilateral nephrectomy in rabbits when the renal nerves are stripped from the pedicle of the remaining kidney. They also reported a high mortality at operation. The present investigation provides an explanation for this high mortality.

The present investigator decided to study the effect of renal denervation while attempting to obtain a method of testing his original hypothesis, namely that, since the kidney expends 80% of its energy in reabsorbing sodium ions, the stimulus to renal hypertrophy must in some way be associated with the maintenance and control of sodium levels. This hypothesis has been strengthened considerably by the work of Devenyi (1964) who found a 40-fold increase in mitotic index in rats given saline drinking water plus an intraperitoneal injection of Antidiuretic Hormone, and by the demonstration that aldosterone increases renal RNA synthesis (Castles and Williamson, 1965).

The most obvious way to prove or disprove this hypothesis is to extract and purify the factor or factors present in the plasma of a nephrectomized animal, whose action on protein and RNA synthesis was illustrated in the experiments described in Section 3.3, and then to determine, using isotopically labelled Na *in vitro* or *in vivo*, whether this factor has direct action on the control of sodium in the

renal tubules or whether it produces an alteration in RNA metabolism similar to that described by Malt (1969) in unilaterally nephrectomized animals.

Unfortunately the facilities for such a study were not available during the course of this work. Another approach was adopted; it was recognised that this approach, even if it gave positive results, would not be a definitive work, but might add even greater substance to the hypothesis that the process of compensatory hypertrophy in the kidney is intimately connected with the control of sodium metabolism.

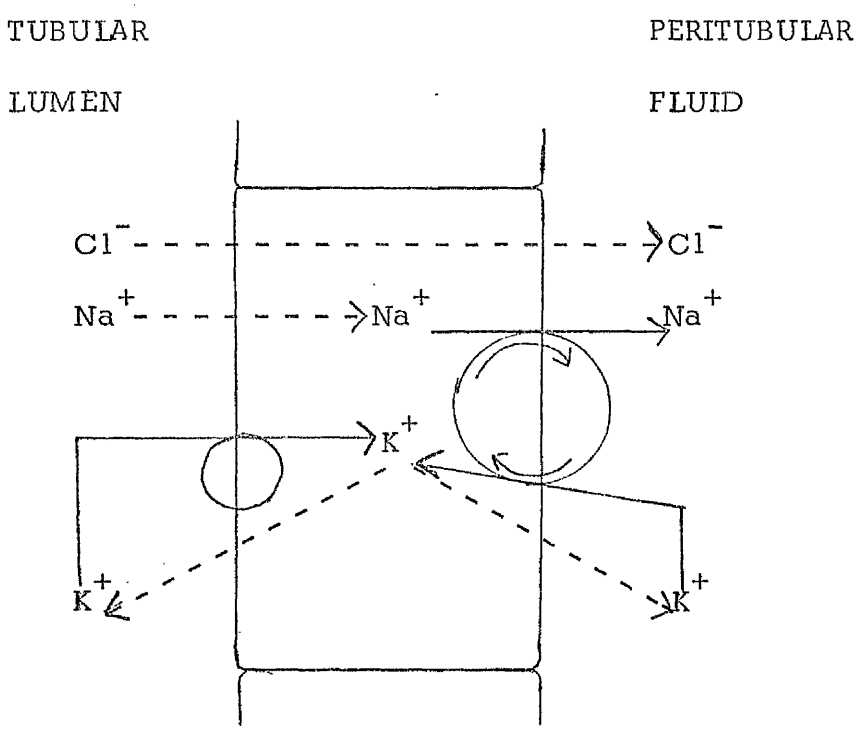
The aim of the experiments described in this section of the work was to increase the amount of sodium reabsorbed by the kidney and to determine whether this increase in reabsorbed sodium was associated with an alteration in renal growth. It is in fact very difficult to increase renal sodium reabsorption in the intact animal for the levels of sodium in blood and extracellular fluid is maintained within very narrow limits. In order to attempt to obtain a method of causing an increase in tubular sodium reabsorption, one must attempt to understand the factors which control sodium reabsorption. This is a field in which there is much activity at present, and also a fair degree of controversy.

Sodium is the principal solid of the ECF, the volume and osmolality of which are closely related to the amount of sodium it contains. It is generally considered that 80% of the sodium which is filtered is reabsorbed together with chloride and bicarbonate in

the proximal tubule. The reabsorption of sodium ions is active, i.e. requires the expenditure of energy; the exact mechanism is unknown, although it is believed to involve the enzyme Na-K activated ATPase and to be associated with the excretion of hydrogen and potassium ions.(de Wardener, 1967). A postulated mechanism is shown in Fig. 17.

There are many factors involved in the control of sodium levels in the body. A rise in the GFR is associated with greater reabsorption of sodium; the amount of sodium excreted does not change as the GFR increases. It seems likely that a sudden rise in GFR produces a sudden rise in pressure in the proximal tubule and that this gives an immediate increase in sodium reabsorption (Rector and Brunner, 1966). Further, McDonald and de Wardener (1965) claim that a sudden expansion of the blood volume causes release of a hormone, known as Third Factor, which inhibits sodium reabsorption, and that this system is in some way related to the system of control of blood pressure which has attracted so much attention recently, i.e. the Renin-Angiotensin-Aldosterone System, which is summarized in Fig. 17.

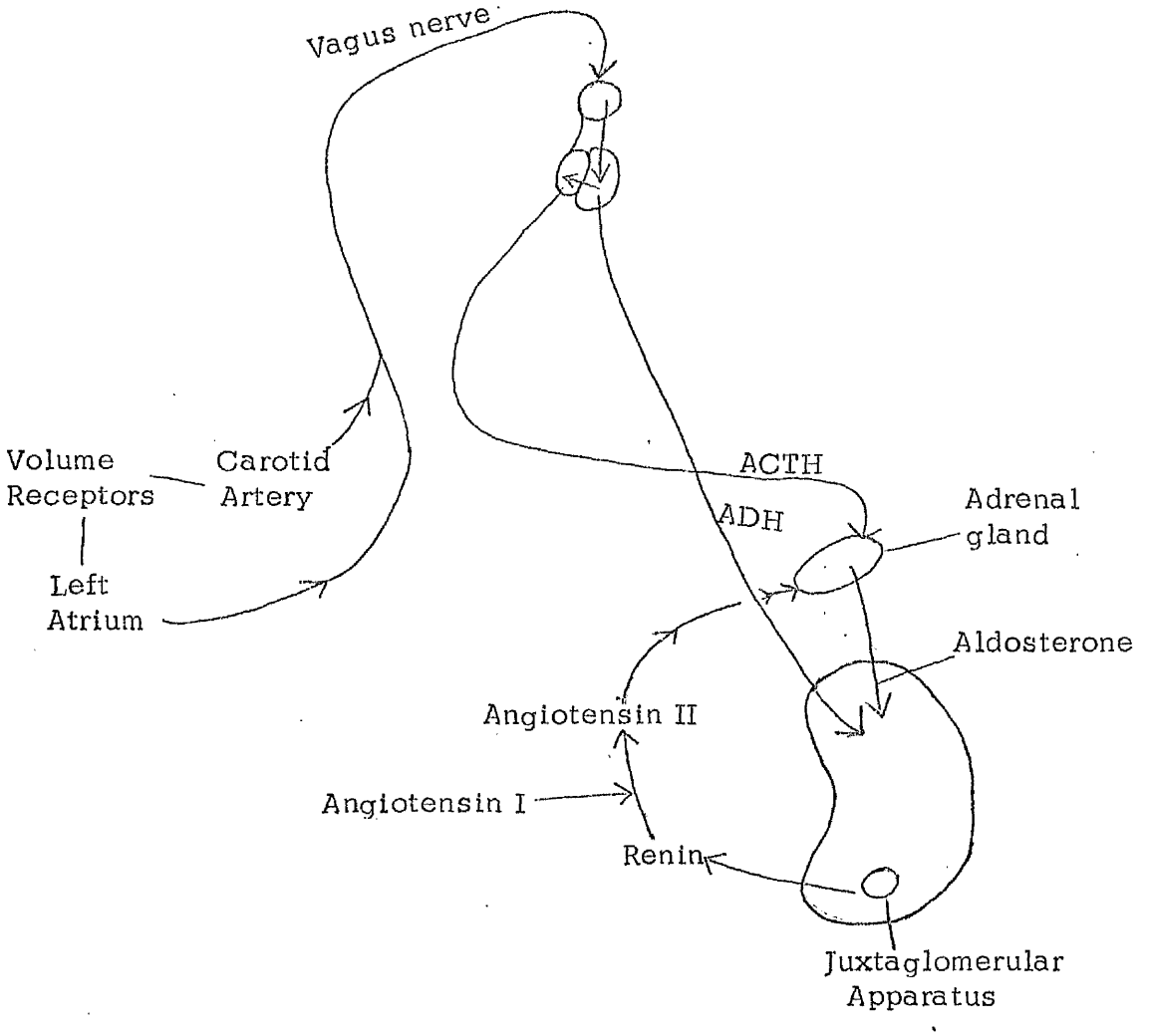
FIG. 16



Schematic representation of ion transport in the proximal tubule.

- - - - Broken lines indicate passive transport

FIG. 17



The Renin-Angiotensin-Aldosterone System

The exact mechanism of renin formation and release is unknown but there is strong evidence that it is formed under conditions of low sodium content in the distal tubule and that its release is dependent on several factors, one of which is the nerve supply to the kidney. There is an inverse relationship between plasma renin and plasma sodium levels (de Wardener, 1967) and renal denervation lowers the plasma renin activity (Vander, 1967). Thus we see a complex relationship between sodium, blood pressure, GFR and body water and blood volume. Masson (1969) found that constriction of the renal artery produced atrophy of the kidney on the affected side and hypertrophy of the contralateral kidney. Now, unilateral renal artery stenosis produces hypertension by causing excessive renin liberation from the kidney. It seems possible that the two factors are related, though not necessarily so as unilateral renal artery stenosis could produce a degree of renal atrophy equivalent to a partial nephrectomy.

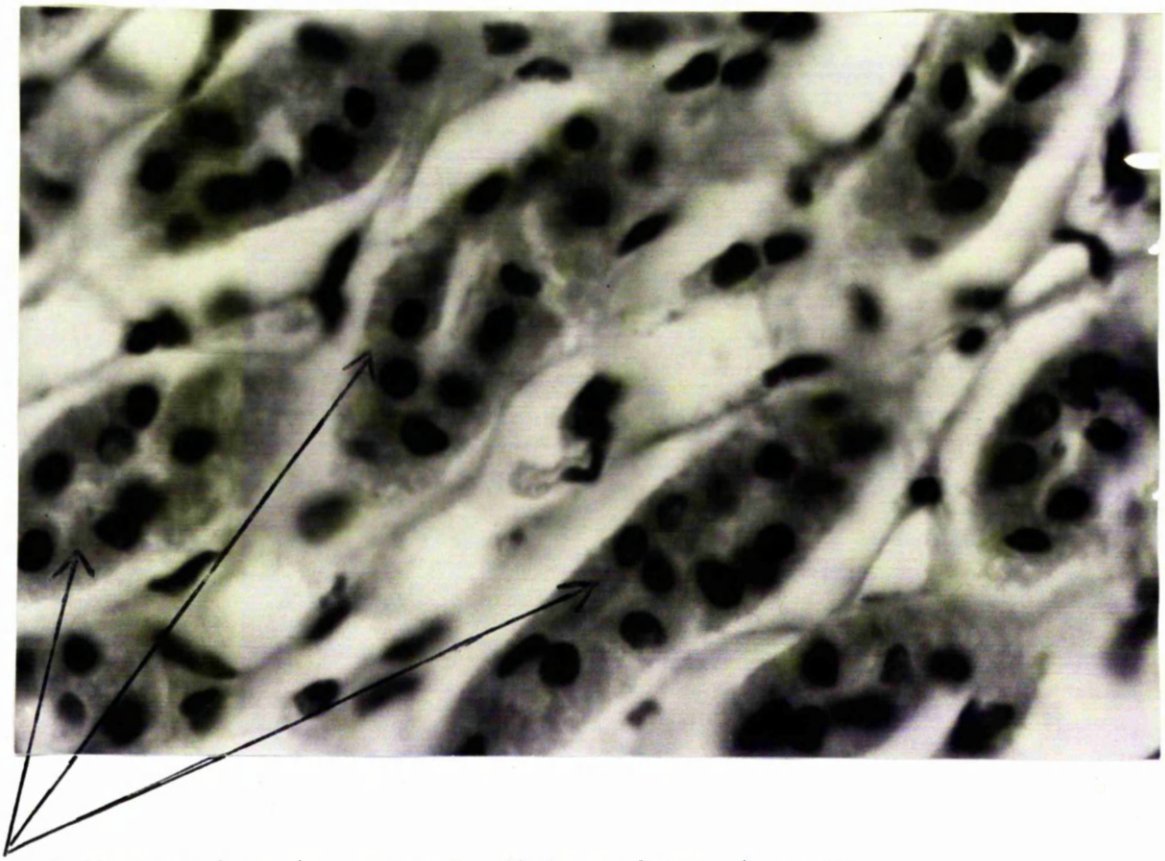
However, since renal denervation reduces renin levels, it might be reasonable to suppose that it increases plasma sodium. If this is the case, it might provide an explanation for the phenomenon described by Allen and Mann. Consequently, it was decided to perform a series of experiments on rabbits to determine whether renal denervation would in fact increase the response to unilateral nephrectomy. Three experiments were performed. One animal did not survive the operation; it did not recover from the anaesthetic. The animals were prepared and operated on as described in Section

2. Only a brief summary of the results is given here as the procedure was found to give surprising results, which might explain the high mortality found by the workers who previously investigated this technique.

Fig. 18 shows a section of the left kidney from a rabbit sacrificed 36 hours post-operatively. The basophilic stippling is caused by excessive intracellular deposition of calcium. Fig. 19 shows a graph of changes in plasma sodium, serum calcium and PCV levels during the first 4 post-operative days. Serum calcium and PCV fell to 50% of normal in the first 2-3 days and then rose slightly; the plasma sodium level was maintained at 20% above normal. However, in view of the pathological changes occurring in the kidney remaining after unilateral nephrectomy, the experimental procedure was not further investigated as to its effect on the response to unilateral nephrectomy.

The explanation for the changes is not clear. They may have occurred due to denervation or due to renal failure induced by the operation, perhaps by removal of the lymphatics along with the renal nerve supply. The change in serum calcium bears some relationship to the condition of renal osteodystrophy which sometimes occurs as a complication of chronic renal failure in man, but as the rabbit normally has a serum calcium level twice that of man no exact parallel can be drawn. An explanation was however obtained for the high mortality reported by Allen and Mann (1935).

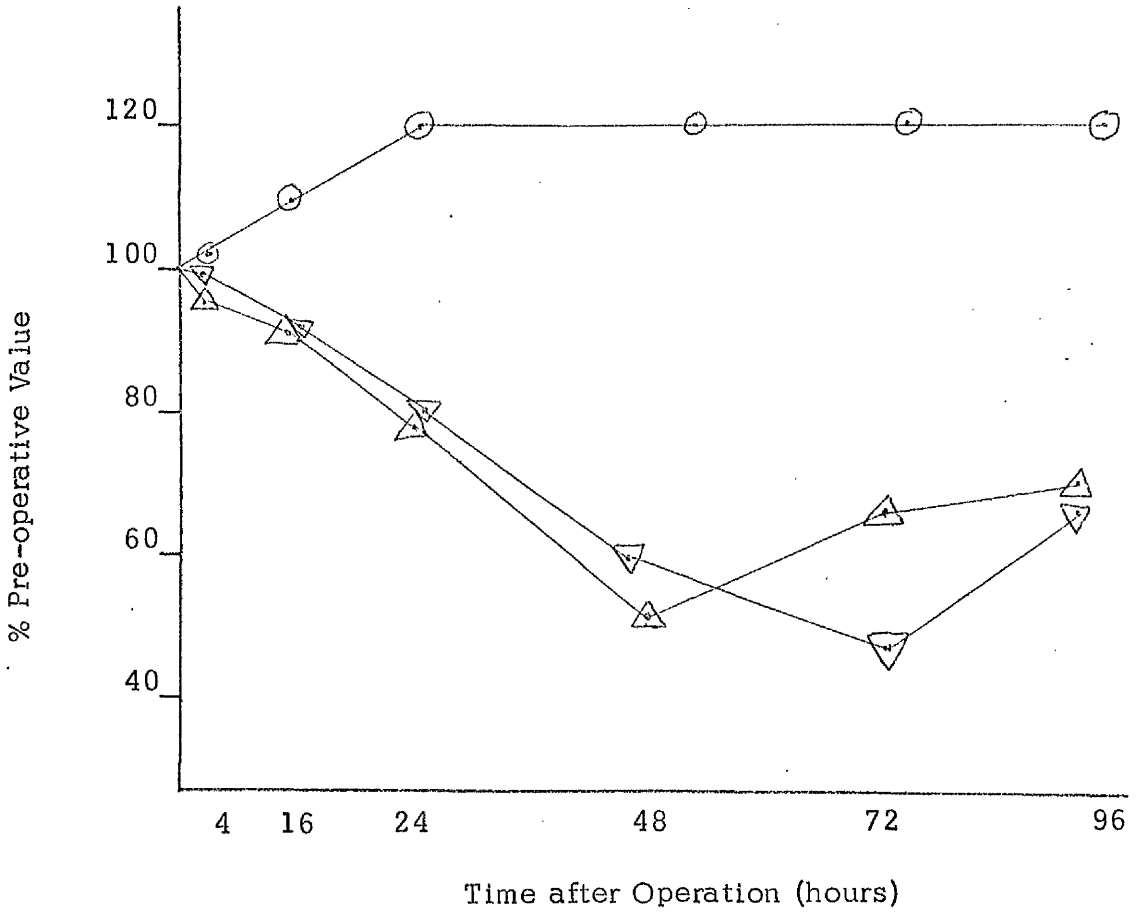
FIG. 18



Basophilic stippling due to intracellular calcium deposition

Photomicrograph of left kidney (x 680)

FIG. 19



The effect of right unilateral nephrectomy and left renal denervation on the plasma sodium, serum calcium and PCV in the rabbit.

⊙ Plasma sodium

▽ Serum calcium

△ PCV

SECTION 4

DISCUSSION

The present investigation was undertaken in an attempt to discover some method which could be used to define the exact mechanism of the control of kidney growth in the renoprival state. Consequently, five types of experiment were performed :

1. Investigation of relationship of the normal kidney weight to other parameters.
2. Investigation of any changes which might occur in the physiological and biochemical state of the rat following unilateral nephrectomy.
3. Investigation of the response of the kidneys to the oral and parenteral administration to the rat of substances which might be thought to promote or inhibit renal growth.
4. Investigation of the hypothesis that compensatory renal hypertrophy is initiated or controlled by an agent or agents which circulate in the blood.
5. Investigation of techniques reputed to increase the compensatory growth which occurs after unilateral nephrectomy.

1. The Normal Rat Kidney Weight

The best correlation found was that between the dry weight of the kidney and the liver weight. This was better than the correlation between the dry weight of the kidney and the dry weight of the liver or the correlation between the dry weight of the kidney over the wet weight of the liver. Halliburton (1966) found evidence of a slight

decrease of isotope incorporation into liver RNA following unilateral nephrectomy. It might be rewarding to study the function of the liver following unilateral nephrectomy.

2. Physiological and Biochemical Changes

No consistent alteration in plasma sodium levels was obtained; no significant alteration in acid-base balance was found; no change in blood ammonia levels were noted. Melvin (1969) found that the excretion of phenolsulphthalein (PSP) was unaltered for 48 hours and that plasma creatinine levels were elevated in the first 48 hours after unilateral nephrectomy. This suggests that functions essential for the animal's well-being are co-ordinated by a compensation which may be renal or extra-renal while those which are non-essential for survival, e.g. PSP clearance or creatinine excretion, are dependent solely on renal growth for their modification. The present investigator believes that as well as hypertrophy of the surviving kidney a degree of extra-renal compensation by the rest of the animal occurs when one kidney is removed. This could be anticipated on grounds of logic, for the more essential a function, the less likely is it to depend on the total functioning presence of a pair of organs.

However, a marked change in the normal plasma glutamine/glutamate ratio was found following unilateral nephrectomy. The explanation for this is not obvious. Since it was not found in the control animal, it would appear not to be due to trauma or anaesthetic administration. Since the % increase in the glutamate level was

approximately the same as the % decrease in the glutamine level, it is reasonable to suppose that it represents an increased conversion of glutamine to glutamate. This might result either from an alteration of kidney or liver metabolism or to an alteration in muscle metabolism or indeed to the presence of the enzyme glutaminase in the blood.

The enzyme amidotransferase was assayed in the kidneys of unoperated, sham operated and right unilaterally nephrectomized animals. No differences between the groups were found when the results were analysed by student t test. However, the groups were small and it was felt that the results did show that the activity in the kidneys from the sham operated animals tended to be lower than that of the other groups. This, if it is the case, would suggest that sham operation depresses amidotransferase activity and that this depression is overcome in the kidney remaining after unilateral nephrectomy. This would correlate well with the work of Van Pilsum (1967a) who found that starvation depresses kidney amidotransferase activity and that the depression is probably due to the high concentration of creatine in the blood of starved animals exerting an inhibitory effect on amidotransferase. This has also been reported by Walker (1959 and 1961).

The main conclusion to be drawn from this section of the work is that very little change occurs in the rat which has undergone unilateral nephrectomy. The exception to this is the marked alteration in the glutamate/glutamine ratio found 14 hours after unilateral nephrectomy. The significance of this is not clear as yet.

3. The Promotion and Inhibition of Renal Growth by Dietary and Parenteral Means

The various dietary methods previously used to stimulate renal growth were described in Section 1. In the present investigation, some of these were used again (gelatin, glycine, acid, sodium chloride and potassium chloride). Another amino acid was investigated (arginine) and the effects of dietary sodium citrate and potassium citrate, and sodium acetate and potassium acetate were studied; in addition the effects of repeated injection of a hypertonic urea/saline mixture were reported.

The repeated injection of the hypertonic urea/saline mixture produced no effect on the kidney weight or composition in the intact animal and did not alter the growth response of the kidney remaining after unilateral nephrectomy. This would suggest that the reports that ureteroperitoneostomy (Fortner and Kiefer, 1948) causes hypertrophy of the kidney might be due to some complication of operation, e.g. infection rather than to the extra load of urea or sodium to be excreted.

It was found that gelatin produced a 40% increase in kidney weight, a 25% increase in kidney RNA, a 22% increase in kidney protein and a (non-significant) 7% increase in kidney DNA. These figures compare very well with the composition of the kidney remaining 48 hours after contralateral nephrectomy, as reported by Halliburton (1966). However, the weight increase of 40% is twice that which occurs 48 hours after unilateral nephrectomy and in

addition the mean cell size is increased by 20% by feeding a diet high in gelatin for 6 days and the mean RNA/100 mg and protein/100 mg are slightly (non-significantly) reduced whereas at 48 hours after unilateral nephrectomy the mean cell size is unaltered and the concentrations of RNA/100 mg and protein/100 mg are increased significantly (Halliburton, 1966). This suggests that in gelatin-induced renal hypertrophy some other substance is accumulating within the renal tubular cells. This might be water, and it would be interesting to perfom wet and dry weight analyses of the kidneys from animals fed a diet containing 30% gelatin. The mechanism of gelatin-induced hypertrophy of the kidney is not clear. It might exert its effect via its chemical composition (glycine is the most abundant amino acid), or via its ability to act as a diuretic. If gelatin causes an increase in GFR and an increase in flow rate down the tubule, one can envisage the possibility that intracellular deficiency of substances normally reabsorbed might induce extra RNA and protein synthesis either at the normal site of reabsorption or lower down the tubule.

The investigation confirmed the findings of Halliburton and Thomson (1967) and Moore (1967) that a diet containing 8% glycine also induces a degree of renal hypertrophy. The extent of the hypertrophy was considerably less than that found by these workers. In the present study, glycine induced 15% increase in renal weight and 6% increase in RNA (when left kidney RNA is considered). Halliburton and Thomson (1967) report 25% increase in renal weight,

an increase in RNA/DNA and an increase in protein/DNA. This might have resulted from differences in the generations of rats used or from some difference in the glycine used. If the response were due to some contaminant of glycine, this would explain the differences between the two studies; they were performed in the same laboratory. It is interesting that glycine also produced a 20% increase in mean cell size. This is similar to the type of growth produced by a diet high in gelatin, though it is of lesser magnitude. It does suggest that the mechanism of glycine-induced hypertrophy of the kidney is similar to that of gelatin-induced hypertrophy of the kidney.

The mechanism of glycine-induced renal hypertrophy is not clear. Glycine is metabolised to serine after accepting a one-carbon unit (folic acid which on injection also causes renal hypertrophy is involved in this process) or is utilized in porphyrin synthesis or in creatine synthesis. It is possible that one of these mechanisms is responsible for the renal growth; if this were the case, one would expect a diet containing 8% glycine to have the same quantitative effect as a diet containing 30% gelatin and this is not the case. The effect could also be due to some contaminant of gelatin and glycine or to the ability of these substances to increase the GFR and to cause an osmotic diuresis. Further work should be directed at a study of various components of the kidney cell during renal hypertrophy induced by glycine and gelatin.

A diet containing 22% L-arginine HCl produced a significant increase in renal weight and a highly significant increase in renal weight relative to body weight. In addition, a fall in liver weight relatively greater than the fall in body weight was noted. The increase in renal weight was certainly due to the HCl component of the molecule which by inducing a chronic metabolic acidosis caused renal hypertrophy. The greater fall in liver weight relative to body weight might also have been due to HCl, but could have occurred due to some toxic effect of arginine. A diet containing 18% L;arginine free base was also studied for its effect on kidney weight and body weight, though its effect on liver weight was not recorded. This form of the amino acid produced a small (insignificant) increase in renal weight, a small (insignificant) decrease in body weight but a highly significant increase in renal weight relative to body weight. To evaluate this finding would require a study of the composition of the kidney (and of the liver) in animals fed arginine free base. This is of course a basic amino acid and the effect may occur because of this property or because arginine in the concentration used in the diet is toxic to the rat.

The addition of an excess of sodium chloride to the diet (2% by weight) produced no effect on kidney weight, nor did the addition of an equimolar quantity of potassium chloride. Neither of the salts had an effect on liver weight or body weight. This suggests that the handling of these salts is absolutely independent of kidney weight, which seems unlikely in the case of sodium. What seems

more likely is that sodium metabolism is so finely controlled that no upset can be produced simply by feeding an increased amount of NaCl.

The addition of potassium citrate (in an amount equimolar with the diet containing potassium chloride) to an otherwise unaugmented diet and to a diet further supplemented with 8% glycine revealed an interaction between glycine and potassium citrate. Thus, potassium citrate induced a 11% increase in renal weight in animals fed an otherwise normal diet and increased the increase in renal weight induced by glycine. The compositions of the kidneys from the animals in this experiment reveal that potassium citrate interacting with glycine causes a reduction in the total RNA of the kidney and produces an increase in RNA/DNA ratio. Further work is necessary, but it appears that potassium citrate causes increase in kidney weight, a reduction in body weight and liver weight, a decrease in RNA and an increase in mean cell size. The increase in RNA/DNA ratio suggests that when glycine and potassium citrate are both present the total kidney DNA is reduced. Thus, potassium citrate probably produces an increase in cell size (perhaps due to water) coupled with a degree of atrophy of the kidney and some loss in body weight.

Potassium citrate is an alkalinising agent and the effect could be mediated via this property. This would suggest that growth and atrophy are processes which occur in response to the animals' environmental or dietary state and suggests that the stimulus to renal hypertrophy might be multifactorial in origin.

The effect of sodium citrate was also studied; its effect on renal mass was similar to that of potassium citrate, i.e. it interacted with glycine to cause an increase, but it does not cause any reduction in body weight. The reduction in body weight was therefore caused by the K^+ ions. The same effects were noted with sodium acetate and potassium acetate; potassium acetate produced a marked reduction in body weight and a relative increase in renal weight. Sodium acetate gave an increase in renal weight and did not affect body weight. The further evaluation and explanation of these phenomena requires a study of kidney weight and dry weight analysis and kidney and liver compositions in animals fed sodium citrate, potassium citrate, sodium acetate and potassium acetate.

The effect of varying the quantities of fat in the diet was also studied. A high fat diet produced no alteration in body weight, kidney or liver weight compared to a control diet. However, a high fat-protein-free diet, while producing no alteration in kidney weight, increased liver weight relative to body weight. This shows that an increase in gluconeogenic demands have no effect on kidney weight. The increase in liver weight requires further evaluation. It may have been due to accumulated fat or to some degree of hypertrophy of the liver.

4. The Humoral Theory

While accepting that a tissue which is preserved artificially on a micropore filter is environmentally removed from its natural state, the present investigation showed that there is the gravest suspicion that there is an agent or agents in the blood of nephrectomized rats which stimulates the growth of kidney cells.

The magnitude of the response obtained is dependent on the concentration of the factor, relative to the number of target cells. In lower relative concentration of plasma it caused a 2-fold but not statistically significant increase in protein and RNA metabolism as measured by isotope incorporation. This response was magnified by decreasing the number of target cells and when one-tenth of the original cells were used, there was a 14-fold increase in isotope incorporation into protein, and RNA, and a 15-fold increase in incorporation into DNA, as compared to the effect of plasma from sham operated animals. Thus, the magnitude of the effect is proportional to the relative concentration as is the qualitative effect produced, i.e. cell division occurs when the increase in protein and RNA is large. This is the same as the situation in compensatory renal growth in vivo (Halliburton, 1966).

This result also explains the differing results obtained by previous workers using cross-circulation and sera transfer techniques. It would obviously be extremely difficult to transfer sufficient serum or blood from one animal to another in order to produce a result which could satisfactorily be measured in the whole animal. The great

advantage of the tissue culture technique is that, instead of increasing the amount of test substance to which the target tissue is exposed, one can vary the amount of target tissue, thereby requiring a smaller quantity of test substance.

What might this humoral factor be? Is there a single factor or are there several components? The present investigation does not answer these questions, although inference can be drawn from this and other investigations. The experiments using dietary agents suggest that growth and atrophy of the kidney can occur in response to a number of agents. The present investigator believes that compensatory renal hypertrophy occurs as a response to many agents. Possibly every substance excreted or reabsorbed actively by the kidney is directly or indirectly responsible for stimulating growth of the enzyme system which handles it.

However, the kidney expends most of its energy in reabsorbing sodium. It is reasonable to suppose that the control of sodium and water metabolism is specially linked to the control of compensatory renal hypertrophy. Moreover, the work of Devenyi (1964) on the effects of injection of ADH coupled with saline administration lend credence to this view. It is reasonable to hypothesise that a major part of the humoral factor's action might be derived from some effect on sodium and water metabolism. ADH is, of course, also a pressor agent, though there is no evidence that its effect is obtained via some action on the blood pressure, although Masson

et al. (1969) have suggested that blood pressure control and kidney growth are related, and Goss (1965) found an increase in renal growth in adrenal regeneration hypertension.

It is also possible that the humoral agent is a promoter or inhibitor of renal growth, the concentration of which is affected by removal of one kidney and which acts directly on the renal nucleic acid, independent of any upset in the metabolism of sodium or any other substance. Such a promoter would be extrarenally produced and its effective concentration increased by removal of 50% of its target tissue; an inhibitor could be produced intrarenally and dependent for its action on its concentration in the blood supplying the kidney. This might seem to be suggested by Malt's work on nucleolar RNA which increases its isotope turnover within minutes of contralateral nephrectomy. However, the function of nucleolar RNA has not been accurately defined; until its function is known, no definite conclusions can be drawn from Malt's excellent work.

There are two arguments against the serum factor being activated by loss of renal tissue per se. Firstly, the kidney is a very specialized organ and handles substances whose CONCENTRATION must be kept within very narrow limits. It would be more logical for the renal mass to be linked to the body's requirements with respect to these vital functions; this was shown to be the case in this study, c.f. the response to a diet containing potassium citrate. Secondly, if compensatory growth occurred as a response to loss of renal tissue per se, one might expect an agent which acted directly on renal DNA

in order to make good the loss, i.e. one might expect a hyperplasia; this would allow less fine control of renal function while the process of compensatory growth was occurring.

It is the opinion of the present investigator that compensatory growth of the kidney is, in large part, stimulated by a humoral agent which owes its action and relative or actual existence to some disturbance of sodium and water metabolism induced by removal of one kidney. This hypothesis is dependent upon the premise that the changes in Hn RNA metabolism, the labelling of ribosomes and the production of membranous whorls described by Malt (1969) are indeed related to the quantitative evidence of hypertrophy obtained later. It is also dependent upon the assumption that the renal growth produced by injection of ADH and saline is in some way, not necessarily directly, related to the events occurring following unilateral nephrectomy.

There are numerous agents which might be responsible for the compensatory growth or else the effect might be due to an as yet undiscovered substance. The more obvious possibilities are ADH, perhaps aldosterone, Third Factor, adenyl cyclase or ribonuclease. One of these agents, or another undiscovered one, or a combination of several might set in motion a chain of events which would lead to RNA and enzyme synthesis, which would in turn reduce the strength of the stimulus, but which would not require the influence of the stimulus for their own control. Cell division would occur when the optimal cytoplasmic/nuclear ratios were exceeded. The

advantage of this mechanism is that it would result in ordered growth in which increase in cytoplasmic contents would be directly proportional to the demands of the animal.

It is of course similar to the old Work Hypertrophy Theory except that it takes into account the complex mechanisms by which sodium and water balance are maintained. As a theory it is no more or less valid than any other; however, some strength is given to it by the findings of the present investigator that there is a substance or substances in the plasma of nephrectomized animals which promotes the growth of adult rat kidney cells in vitro.

5. Modification of the Response to Unilateral Nephrectomy

In this section of the work the claim that there might be non-specific growth promotional agents was investigated. Paschkis, Goddard, Contarow and Adibi (1959) reported that two-thirds partial hepatectomy exaggerated the growth of the kidney remaining after unilateral nephrectomy. This finding was not confirmed. There was no difference in compensatory growth of the kidney 4 days after unilateral nephrectomy in rats which had a two-thirds partial hepatectomy just prior to unilateral nephrectomy. In addition, the regrowth of the liver was identical to that which would have been predicted at 4 days (Brues et al., 1936).

The claim by Allen and Mann (1935) that renal denervation and contralateral nephrectomy in the rabbit gave an increase over normal compensatory growth prompted a further investigation of the technique.

It was found that renal denervation and contralateral nephrectomy induced a state of acute hypernatraemic, hypocalcaemic nephrocalcinosis and the experiments were not pursued. The explanation for the changes is not clear. The hypernatraemia is probably due to renal denervation, but the changes in serum calcium are not easy to understand. They might be due to renal failure or to some anatomical abnormality, e.g. removal of the lymphatics induced by the operative procedure.

CONCLUSIONS

The removal of one of a pair of kidneys produces only minor changes in the composition of the blood and no changes at all in substances which are toxic or whose level has to be maintained between narrow limits. By various dietary means (glycine, gelatin or acid) it is possible to stimulate the growth of the kidneys in intact animals. It is also possible to produce atrophy of the kidneys using a potassium citrate diet. This suggests that renal mass is not constant even in the intact animal, but that growth and atrophy are processes which go on all the time, depending upon the environmental influences to which the animal is exposed.

This growth must not be seen as a process separate from normal activity, but as an integral part of normal metabolic processes, the exact stimulus to which remains obscure. In the case of compensatory renal hypertrophy, an artificially produced exaggeration of the normal renal growth mechanism, there was evidence that the increased renal

mass resulted from the summation of various component stimuli and that possibly each enzyme system was separately activated. There was, however, evidence of a factor in the plasma of nephrectomized rats which stimulates the growth of adult kidney cells in vitro. This factor may be a single substance which plays a much more important part than any other in stimulating growth in the renoprival state; this would seem reasonable since the kidney is a very specialized tissue.

Future work should be directed towards isolating and identifying this factor and its mode of action, towards studying the changes occurring during the first few hours following unilateral nephrectomy in the RNA of the remaining kidney and towards studying possible alterations in specific components of kidney tissue induced by gelatin, glycine, and sodium and potassium citrate.

SECTION 5

SUMMARY

1. The correlations between total renal weight and other parameters were investigated. The total renal dry weight and total renal water content show fairly good correlation with body weight, liver weight and liver dry weight. The best correlation is that between renal dry weight and liver weight.

2. The physiological and biochemical state of the rat in the early stages following right unilateral nephrectomy was investigated.

(1) There is no consistent alteration in the level of sodium ions in the plasma in the early stages following unilateral nephrectomy when compared to sham operated controls.

(2) There is no consistent alteration in the state of acid-base balance in the early stages following unilateral nephrectomy when compared to sham operated controls.

(3) There is a reversal of the normal plasma glutamate/glutamine ratio 14 hours after unilateral nephrectomy. This has returned to normal by 24 hours after operation.

(4) There is no consistent alteration in the blood ammonia levels in the early stages following unilateral nephrectomy when compared to sham operated controls.

(5) There is no significant alteration in the level of activity of the enzyme amidotransferase in the kidney remaining 2 days after unilateral nephrectomy. There is a possibility that sham operation results in a reduction in kidney amidotransferase activity, and that this reduction is overcome in the kidney remaining after unilateral nephrectomy.

(6) There is no alteration in the level of arterial blood pressure in the first 30 minutes following unilateral nephrectomy.

3. The stimulation and inhibition of renal growth in the intact and nephrectomized rat was investigated by the oral and intravenous administration of substances which might be thought to promote or retard growth of the kidneys.

(1) There was no alteration in total renal weight or in total renal RNA content or in the concentration of RNA per 100 mg. tissue as a result of the repeated intravenous injection of a hypertonic urea/saline mixture. Further, intravenous injection of this substance did not affect the response to unilateral nephrectomy.

(2) There was no alteration in body weight, total renal weight or liver weight as a result of the administration for 6 days of diets containing 2% sodium chloride or an equimolar quantity of potassium chloride.

(3) There was a marked increase in renal weight and an alteration in renal composition as a result of the administration for

6 days of a diet containing 30% gelatin. There was a 40% increase in total renal weight, a 25% increase in total renal RNA and a 22% increase in total renal protein. The quantitative changes in renal nucleic acids and protein are remarkably similar to those which occur 48 hours after unilateral nephrectomy but the 40% increase in renal weight is twice what would be expected at this time and in addition gelatin causes a 20% increase in mean cell size and a small decrease in RNA/100 mg and a small decrease in protein/100 mg. This does not occur following unilateral nephrectomy and suggests the accumulation within the cells of some other substance. It was thought that the effect of gelatin could be mediated either by physical or its chemical properties.

(4) There was a significant increase in renal weight and an alteration in renal composition as a result of the administration for 6 days of a diet containing 8% glycine. There was a 15% increase in total renal weight and a 6% increase in left kidney RNA but no increase in total renal protein or DNA. In addition, there was a 20% increase in mean cell size, a small decrease in RNA/100 mg and a small decrease in protein/100 mg. This is a similar pattern, though a much smaller response than that obtained with a 30% gelatin diet, and again suggests the accumulation of some other substance within the cell. The conclusion drawn was that glycine which is the most abundant amino acid in gelatin produces its

effect by a mechanism similar to that of gelatin, and that the stimulus might be physical or chemical in nature.

(5) There was no alteration in renal weight as a result of the administration for 6 days of a diet containing 18% L-arginine. However, there was a significant increase in the kidney weight/body weight ratio.

(6) There was a significant increase in total renal weight as a result of the administration of a diet containing 22% L-arginine HCl. There was also a marked fall in body weight and therefore a very great increase in the kidney weight/body weight ratio; liver weight fell relatively more than body weight. The conclusion was that a state of chronic metabolic acidosis had been induced and that this was responsible for the increase in renal weight, but that some part of the fall in body weight was due to the L-arginine which is probably toxic in the high concentration used in these diets.

(7) There was a marked alteration in total renal weight and kidney composition as a result of the administration of 10% potassium citrate in the diet. On the addition of potassium citrate to an otherwise normal diet there was 12% increase in total renal weight and 15% increase in the renal weight/body weight ratio, i.e. potassium citrate caused an increase in kidney weight and a decrease in body weight. When 8% glycine and 10% potassium citrate were given in combination, there was a 27% increase in total

renal weight and a 30% increase in renal weight/body weight ratio. On tissue analysis the two supplements were found to interact to produce a fall in total kidney RNA. Thus, potassium citrate when given alone gave a 13% reduction in kidney RNA; glycine when fed alone gave a 6% increase in left kidney RNA. In addition, the effect of potassium citrate on kidney RNA is greater in the presence of glycine than in its absence. Such an interaction between glycine and potassium citrate also plays a part in producing an increase in RNA/DNA ratio and a decrease in RNA/100 mg, and a decrease in DNA/100 mg. It seems possible that some other factor, e.g. overhydration of the cell or abnormal intracellular electrolyte balance comes into play when glycine and potassium citrate are fed in combination. The greater part of the effect, however, was thought to be due to the alkalinizing property of potassium citrate causing a degree of renal atrophy by acting on the enzyme systems responsible for the excretion of acid. It was found that sodium citrate, in the presence of glycine, produced an increase in renal weight; sodium acetate caused an increase in renal weight, acting singly or in combination with glycine. Neither of these salts affected the body weight. Potassium acetate, while producing no increase in total renal weight, affected body weight so much that there was a large increase in the renal weight/body weight ratio. Thus, the fall in body weight is a property of the K^+ ions.

4. The possible role of humoral agents, circulating in the blood of nephrectomized animals, in stimulating renal growth was investigated using an in vitro system of adult rat kidney cells aggregating on micropore filters.

(1) There was biochemical evidence of viability, measured by isotope uptake into nucleic acids and protein for at least 4 days after initial culture inoculation. As a result of the studies on isotope incorporation at various times after initial culture inoculation it was decided to harvest all future cultures 3 days after initial inoculation, following a 6-hour isotope incorporation.

(2) There was fairly good evidence of a stimulating factor, or factors, in the plasma of animals nephrectomized 36 hours prior to withdrawal of plasma. With 30×10^6 cells exposed to 15% plasma from nephrectomized animals for 7 hours there was non-significant increase in incorporation of ^3H leucine compared to the incorporation into the same number of cells exposed for the same time to 15% plasma from animals sham operated 36 hours previously. With 10×10^6 cells exposed to plasma from nephrectomized animals there was a 2-fold increase in ^3H leucine incorporation which was again insignificant. In neither of the above cases was there any increase in ^{14}C thymidine incorporation which was very low in both controls and test cultures. When 3×10^6 cells were subjected to similar plasma exposition, there

was a 14-fold increase in ^{3}H leucine incorporation and a 15-fold increase in ^{14}C thymidine incorporation in the cultures incubated with plasma from nephrectomized animals. This shows evidence of greatly increased protein, RNA and DNA synthesis. Thus, there is a factor or factors in the plasma of nephrectomized animals which promotes renal growth and whose effect can be quantitated. The magnitude of the response obtained is proportional to the concentration relative to the number of cells; in lower relative concentrations it produces a hypertrophy, i.e. increase in RNA and protein synthesis, while in higher concentration it also promotes DNA synthesis, i.e. promotes a hyperplasia. However, it may be that the number of cells per unit area has by itself some effect on the degree of DNA synthesis and subsequent mitosis.

5. Two of the methods reputed to exaggerate the normal compensatory growth which follows unilateral nephrectomy were investigated :

(1) Simultaneous two-thirds partial hepatectomy produced no alteration in the response to unilateral nephrectomy in the rat at 96 hours. This is felt to disprove the theory of a non-tissue-specific growth promoting mechanism. There was no alteration in the rate of regrowth of the liver fragment as a result of unilateral nephrectomy.

(2) Unilateral nephrectomy plus contralateral renal denervation in the rabbit produced a high operative mortality;

as well as hypernatraemia, hypocalcaemia and acute nephrocalcinosis and a fall in PCV. In the animal which survived the hypernatraemia persisted throughout the 4 days of the experiment, whereas the hypocalcaemia and fall in PCV reached a maximum of 55% below normal at 3 days and thereafter began to minimize. In view of these pathological conditions, the experiments were discontinued and no conclusions drawn regarding the previously reported increased response obtained by renal denervation and contralateral nephrectomy.

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