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Chemical Aspects of Kidney Growth.

by

Ian W. Halliburton, B.Sc.

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C O N T E N T S

SECTION 1.

I N T R O D U C T I O N.

I N T R O D U C T I O N.

1. General introduction.

The kidney is an organ of the body which is essential for survival. Total nephrectomy is followed by death within a few days. Removal of one kidney, however, is perfectly compatible with a normal life span, at least in man (Hinman, 1943). It has long been known that if one kidney is diseased to the point of atrophy, its partner shows a corresponding hypertrophy (Smith, 1951). A similar hypertrophy can be achieved experimentally by simple removal of one kidney or by removal of one kidney and a portion of the other (Markowitz, 1954; Goss, 1964). This process of compensatory renal hypertrophy, as it is generally called, has been studied by many workers since Rayer first described it in 1841 (Braun-Menéndez, 1952).

2. Structural aspects of compensatory renal hypertrophy.

1. The increase in kidney weight.

Many observations have been made on the increase in the weight of the remaining kidney after unilateral nephrectomy. The most important of these are shown in Table 1. Despite the inevitable biological variation, there appears to be general agreement about the rate of the hypertrophy. In the rat - the most studied species - growth seems to start within one or two days of the operation. By about 17-40 days, the kidney is about half as large again. At the longest time intervals included in experimental studies, the kidney is about 70% heavier than it was before the operation ie. the loss

Table 1.

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

Investigator(s)	Date	Species Studied	Body Weight	Observed Weight Increase In Remaining Kidney
Kurnick	1955	Rat	160-240g.	3% at 1 day 40% at 18 days
Royce	1963	Rat	130-200g.	12% at 2 days
Becker & Ogawa	1959	Rat	180g.	11% at 3 days 35% at 10 days 44% at 17 days
Miyada & Kurnick	1960	Rat	None given	30% at 4 days
Fautrez, Cavalli & Pisi	1955	Rat	None given	30% at 7 days
Montfort & Perez-Tamayo	1962	Rat	79-159g.	22% at 10 days 39% at 20 days
Threlfall, Cairnie, Taylor & Buck	1964	Rat	None given	35% at 21 days
Schaffenburg, Masson & Corcoran	1954	Rat	150g.	Significant at 1 day 27-78% at 32 days
Addis & Lew	1940	Rat	None given	70% at 40 days
Reiter	1965	Mouse	Various	23% at 14 days
Straube & Patt	1961	Mouse	Various	40% at 14 days 50% at 26-38 days
Fajers	1957	Rabbit	1500-2820g.	Significant at 1 day 20% at 10-20 days
Addis, Myers & Oliver	1924	Rabbit	None given	58% at 15-33 days 66% at 106-126 days

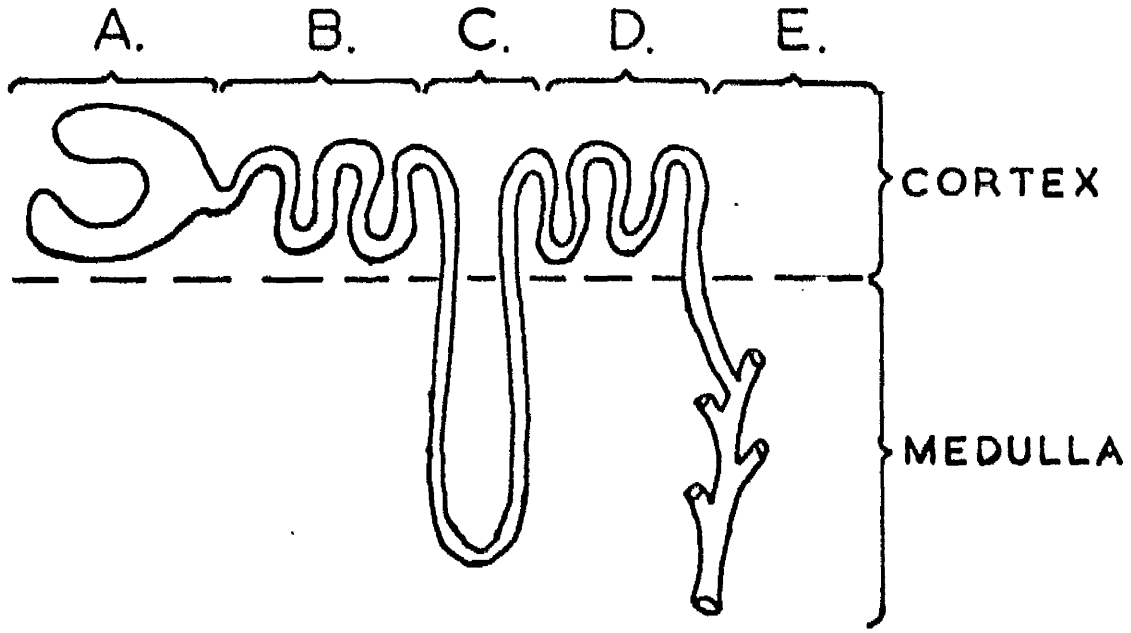
of kidney tissue has not been completely made good. The observations in mice and rabbits are far less extensive, but those shown in Table 1 would be consistent with the assumption that the rate of the process is about the same as in the rat. Although Smith and Moise in 1927 reported an increase in the water content of the kidney remaining after unilateral nephrectomy, more recent experiments (Montfort and Perés Tamayo, 1962; Straube and Patt, 1961) have failed to confirm this, and the changes in wet weight would appear to parallel corresponding changes in dry weight.

2.2. Histology.

The unit of structure in the kidney is the nephron, consisting of the glomerulus with its associated tubules (Fig. 1). The increase in weight of the surviving kidney after unilateral nephrectomy might be attributable either to an increase in the number of nephrons or to an increase in the size of the individual nephrons or to both. Arataki (1926), Saphir (1927) and Shiels (1927) estimated the numbers of nephrons in rat kidneys by counting the glomeruli in histological sections. They reported independently that the number did not increase in the surviving kidney after unilateral nephrectomy. More recently, Hiramoto, Bernecky and Jurand (1962) have prepared, in rabbits, an antiserum to rat kidneys. By injecting this into rats they labelled all the existing glomeruli. The rats were then unilaterally nephrectomized. Seven months later they were killed and their remaining kidneys examined. Although considerable compensatory

FIGURE 1.

DIAGRAMMATIC REPRESENTATION OF THE KIDNEY NEPHRON.



A. GLOMERULUS.

B. PROXIMAL TUBULE.

C. LOOP OF HENLE.

D. DISTAL TUBULE.

E. COLLECTING TUBULE.

hypertrophy had occurred, it was shown (by the indirect fluorescent antibody technique) that all the glomeruli were still labelled. Therefore no new glomeruli had been formed during the hypertrophy.

The increase in size of the kidney after unilateral nephrectomy must consequently involve growth of the individual nephrons rather than increase in their number. Although Moll (1955) could find no increase in glomerular or tubular diameters in mouse kidneys after unilateral nephrectomy, many other workers have reported size increases in these structures in rats and in rabbits (Boycott, 1910; Oliver, 1924; Arataki, 1926; Saphir, 1927; Morrison, 1962). Arataki (1926) also found an increase of the supporting tissue between the tubules.

2.3. Mitotic index.

Since compensatory renal hypertrophy presumably involves some increase in cell number, it seems reasonable to expect that this increase will manifest itself in a transient outburst of mitotic activity. In normal adult kidney, as in most tissues of normal mature animals, the rate of cell division is very low. The mitotic frequency has been reported as 15 to 40 per 100,000 nuclei by Franok (1960) and as 24 per 100,000 nuclei by Goss and Rankin (1960). In the proximal convoluted tubules of young adult rats McCreight and Sulkin (1959) reported the mitotic frequency as 100 per 100,000 nuclei, decreasing to 25 per 100,000 nuclei in senile rats. Using colchicine to arrest mitoses in mice, Argyris and Trimble (1964)

found that mitotic frequency in the mouse kidney was twice as high in males as in females. Mitotic frequency in the normal kidney is known to vary with the time of day. Blumenfeld (1942) and Williams (1961) are in agreement that it reaches a maximum between 2 and 4 p.m., but whereas Blumenfeld (1942) places the minimum at 10-12 p.m., Williams (1961) puts it at 6-8 a.m. Zakharov (1961) has also found mitotic frequencies high in the morning and low at night. This type of diurnal variation is common to other tissues, notably the liver (Jaffe, 1954). Mitoses have been shown to be most numerous in the proximal tubules, least numerous in the distal tubules and collecting ducts, and intermediate in the ascending limbs of the loop of Henle (Williams, 1961). The mitotic activity therefore seems to be greatest at the glomerular end of the tubule.

Following unilateral nephrectomy there is an increase in the mitotic activity in the surviving kidney. Generally, in the rat and in the mouse this does not develop until the second day after the operation and does not persist for long (Rollason, 1949; Ogawa and Sinclair, 1958; Franek, 1960; Goss and Rankin, 1960; Rosen and Cole, 1960; Argyris and Trimble, 1964). On the other hand, Sulkin (1949) and McCreight and Sulkin (1959) have reported a maximum at 3 days in the rat and Semenova (1961) found no increase in the mouse before the seventh day. If only part of one kidney is removed, mitotic activity in the residual fragment and in its intact partner, is also reported to reach a maximum at 2-3 days (Saetren, 1956;

Semenova, 1961). Finally, if all of one kidney and half of its partner are removed, once again mitotic activity is maximal at 2 days (Steuart, 1958). Some investigators have noted two peaks of mitotic activity following unilateral nephrectomy. Ogawa and Sinclair (1958) and Franck (1960) reported one at two days and another on the seventh day. Sulkin (1949) however, reported peaks on the third and tenth days. Williams (1961) recorded the primary mitotic response at 40 hours after unilateral nephrectomy, with a secondary smaller response at 3 to 4 days.

At its maximum, the frequency of mitoses after unilateral nephrectomy in rats is about 3-6 times that found in normal kidneys (Franck, 1960; Goss and Rankin, 1960). In mice, using colchicine to arrest mitoses, Argyris and Trimble (1964) found a sex difference in the magnitude of the response: the increase was 11-fold in males, but only 5-fold in females. McCreight and Sulkin (1959) have suggested that young and old rats may differ; they found a 7 fold increase in young adult rats but only a 5-6 fold increase in senile rats. The mitotic activity after unilateral nephrectomy follows the same pattern as in the normal kidney. It is highest in the cortex, particularly in the tubular cells (Carnot and May, 1938; Ogawa and Sinclair, 1958; Williams, 1961). The outer medulla responds similarly, but to a lesser extent and the inner medulla has considerably lower mitotic activity (Carnot and May, 1938; Ogawa and Sinclair, 1958; Williams, 1961).

3. Chemical aspects of compensatory renal hypertrophy.

1. DNA synthesis.

It is now accepted that in general the DNA content per set of chromosomes is constant in the somatic cells of different tissues of any given species (Vendrely, 1955). Since kidney cell nuclei all have about the same DNA content, they are presumably all diploid (Thomson and Frazer, 1954). The increase in cell number in the surviving kidney after unilateral nephrectomy should therefore be paralleled by an equivalent increase in total DNA content. Mandel, Mandel and Jacob (1950a) reported an increase of 16% in the DNA content of the remaining kidney 15 days after unilateral nephrectomy, rising to 18% at 30 days and 37% at 80 days. Three weeks post-operatively, Threlfall, Cairnie, Taylor and Buck (1964) found a 21% increase in DNA content per kidney and Kennedy (1960) reported a 15% increase at the same time interval rising to 58% after 6 weeks. The age of the rat does not appear to affect the magnitude of the response, for Barrows, Roeder and Olewine (1962) found an increase of about 44% in the DNA content per kidney in both young adult and old rats, 8 weeks after unilateral nephrectomy.

The increase in DNA content is not apparent immediately after the operation. Miyada and Kurnick (1960) were unable to detect any significant change in rat DNA content per kidney in the first 4 days post-operatively, but found a 30% increase between the sixth and sixteenth days. On the seventh day, Lotspeich (1965) reported a 20%

increase in DNA content per kidney although Mandel, Mandel and Jacob (1950a) detected only a 4% increase at this time. In the mouse, Semenova (1961) was able to detect an increase in the total DNA content per kidney 3 days post-operatively, but Straube and Patt (1961) could find no significant increase in renal DNA content even after 5-6 days. These findings are in agreement with the histological observations that the increase in cell number does not get under way until the second or third day after the operation (Section 1. 2. 3).

From cytophotometric evidence it seems quite clear that when a cell is about to undergo mitosis it accumulates the additional amount of DNA required beforehand (Howard, 1956). Consequently a population in which mitosis is frequent should contain a proportion of nuclei with more than the standard quota of DNA. This prediction is borne out in the present instance by experimental observations. Although Kurnick (1955), using chemical analysis of nuclei isolated in bulk, could find no increase in average DNA per nucleus during compensatory renal hypertrophy, Ogawa (1961) using similar methods, found a 10% increase from the third to the fifth post-operative day. ie. at about the time of maximum mitotic activity. Cytophotometric measurements of the relative DNA contents of individual nuclei have shown that a few days after the operation the percentage of nuclei with about twice the normal DNA content was significantly increased (Franck, 1958; Becker and Ogawa, 1959). This increase presumably

represents cells which have doubled their DNA content in preparation for division.

This synthesis of DNA prior to cell division can also be demonstrated by isotope uptake. Using [^{3}H] thymidine injection and autoradiography, Benitez and Shaka (1964) found a significant increase in the number of labelled cells 24 hours post-operatively. The number fell slightly after 48 and 72 hours but it was still significantly greater than in control animals at both these times. Lowenstein and Stern (1963) and Reiter and McCreight (1965a), using the same procedure, have also found increased uptake of the label 2 days post-operatively. Noltenius, Kemperman and Oehlert (1964) on the other hand, reported that the number of labelled cells actually decreased in the first 48 hours but rose from the third post-operative day, the increase lasting until the fifth or sixth day and then disappearing. Lowenstein and Stern (1963) found that the majority of the labelled cells were in the cortex with few in the glomeruli or medulla. Reiter and McCreight (1965a) similarly found that the percentage of labelled cells in the cortex was greater than that in the medulla. These results would agree with the findings of the distribution of cells in mitosis following unilateral nephrectomy (Section 1. 2. 3). The use of ^{32}P uptake as an index of DNA synthesis has given broadly similar results. Simpson (1961a) found that uptake reached a maximum 48 hours post-operatively, declined to a minimum after 8 days but showed a second rise on the ninth post-operative

day. This second rise might correspond to the second peak of mitotic activity found after 7 to 10 days by some histologists (Section 1. 2. 3). Royce (1963) has also shown an increased uptake of ^{32}P 2 days post-operatively.

In summary, therefore, the chemical, cytophotometric and isotopic evidence of DNA synthesis during compensatory renal hypertrophy is quite consistent with the histological observations of mitotic activity.

3.2. RNA synthesis.

It is a general finding that when tissues grow or synthesize proteins for other purposes, their RNA content increases (Brachet, 1955; Belozersky and Spirin, 1955). There is plenty of evidence for an increase in the RNA of the surviving kidney after unilateral nephrectomy, but it is not always easy to decide whether this represents the establishment of machinery to permit growth in a tissue which does not normally grow, or whether it is merely one manifestation of the general increase in kidney substance. For example, Mandel, Wintzerith, Jacob, Perry and Mandel (1957) reported that after unilateral nephrectomy the RNA of the surviving kidney showed an increase before any other tissue constituent. The increase amounted to 20% after 24 hours and 30-39% after 3 days. This early increase would be consistent with the idea that RNA was being accumulated in preparation for the extensive protein synthesis required for subsequent growth. Similarly, three days after removal of approximately $\frac{1}{3}$-$\frac{1}{2}$ of one kidney, Semenova (1961) found

an increase in renal RNA concentration. Seven days after unilateral nephrectomy Lotspeich (1965) has shown a 37% increase in RNA in the surviving kidney. Barrows, Roeder and Olewine (1962) have reported a difference in the magnitude of the response with the age of the animals used. Eight weeks post-operatively the RNA content per kidney had increased by about 45% in young adult rats (12 months old) but only by about 34% in old animals (21 months old).

Because the DNA content per cell nucleus is virtually constant, it is often informative to compare other cell constituents to DNA and thus obtain a picture of average composition per cell. Mandel et al. (1950a) stated that at the end of two months the kidney hypertrophy had reached a plateau, and although the total RNA and DNA contents of the surviving kidney had increased, the $^{RNA}/_{DNA}$ ratio was the same as that for either sham-operated controls or for normal rat kidneys. Barrows et al. (1962) have also found that 8 weeks after unilateral nephrectomy the $^{RNA}/_{DNA}$ ratio in the hypertrophied kidney was the same as in normal kidney, irrespective of the age of the animals. Kurnick (1955) however has shown that the $^{RNA}/_{DNA}$ ratio shows a transient increase in the first few days after unilateral nephrectomy, reaching a maximum about 35% above normal 3-4 days post-operatively and returning to the original level about the ninth day. Seven days post-operatively Lotspeich (1965) reported a 14% increase in the $^{RNA}/_{DNA}$ ratio.

The overall picture therefore, is that, as might be expected,

the RNA content per cell is inflated during the period of most rapid growth, but returns to normal as growth slows down and eventually ceases.

3.3. Protein synthesis.

The available evidence here is more sketchy. The total amount of nitrogen in the remaining kidney, like everything else, increases after unilateral nephrectomy. Mandel et al. (1950a) reported a 39% increase 30 days post-operatively. Kennedy (1960) found a 43% increase after 3 weeks, rising to a 52% increase after 6 weeks. Barrows et al. (1962) found a 44% increase in young rats after 8 weeks but only a 36% increase in old rats at the same time. More recently, Threlfall et al. (1964) reported a 39% increase in total nitrogen 21 days post-operatively. Barrows et al. (1962) found no change in the protein nitrogen/DNA ratio 8 weeks post-operatively. Thus there is general agreement on the size of the increase in protein nitrogen at fairly long time intervals post-operatively. We do not know however whether there is an initial transient rise in protein per cell corresponding to the increase in RNA per cell.

4. Control of compensatory renal hypertrophy.

1. The work hypertrophy theory.

The mechanism controlling compensatory renal hypertrophy is unknown. An obvious possibility is that it is due to the surviving kidney having to carry out the duties normally shared between two. The most obvious function of the kidney is the excretion of urine,

and a number of early investigators tried to find out what might happen if the urine was diverted to some site where it would be reabsorbed. Since it would then have to be re-excreted, this would increase the excretory work the kidneys would have to do.

Hartman (1933), for example, established an anastomosis between the urinary bladder and the distal end of the ileum and found that the kidneys enlarged in the next 6 to 8 weeks, after which there was no further increase in size. With the same object in view he also injected concentrated human urine via a catheter into the ileum of dogs; this caused the death of the majority of the animals within 24 hours. More recently Fortner and Kiefer (1948), using dogs, transplanted a ureter into the duodenum and found that in some of the animals the contralateral kidney hypertrophied. Using the same procedure however, Block, Walkin and Mann (1953) did not find any increase in the weight of the contralateral kidney, although Bollman and Mann (1935) found that hypertrophy of the kidney remaining after unilateral nephrectomy was greatly increased following transplantation of its ureter into the duodenum.

If, instead of being transplanted into the small intestine, the ureter is merely severed, the urine should drain into the peritoneal cavity from which it must be reabsorbed and re-excreted. Mason and Ewald (1965) found that this operation produced an increase in the weight of the kidney on the unoperated side. Goss and Rankin (1960), however, found no significant increase in mitotic

activity in either kidney. Simpson (1961a), who investigated only the kidney on the unoperated side found no increase in DNA synthesis as indicated by ^{32}P uptake. Royce (1963) obtained similar results. He also noted that the operation was always associated with inflammation of the peritoneum. He therefore introduced a talc suspension into the peritoneal cavity of unilaterally nephrectomized rats. This inhibited compensatory renal hypertrophy. It is possible therefore that the failure of the kidneys to hypertrophy after section of the ureter is a result of the associated inflammation. The situation might also be complicated by inflammation and consequent blockage of the severed ureter itself. Presumably however, the effects of such blockage would be similar to those produced by experimental ligation of the ureter. This is followed by hydronephrosis and a marked increase in weight and mitotic index in the obstructed kidney. The other kidney shows a slight increase in weight and mitotic index (Hinman, 1923; Idbohrn and Muren, 1956; Goss and Rankin, 1960; Benitez and Shaka, 1964; Mason and Ewald, 1965). The relevance of these observations is difficult to assess, for not only is the output from one kidney stopped, but the kidney is also distorted by hydronephrosis.

An alternative approach to the problem is to modify the diet in such a way as to give the kidney more work to do. One of the main functions of the kidney is the excretion of urea. The amount to be excreted can readily be varied by varying the protein content

of the diet. Reader and Drummond (1925) fed diets containing 45 or 90% caseinogen for a period of some months to rats and found that, though the animals were otherwise normal, their kidneys had hypertrophied. Osborne, Mendel, Park and Winternitz (1927) found that high-protein diets could produce significant increases in kidney size in the rat in as little as 8 days. MacKay, MacKay and Addis (1927a) fed diets containing 18%, 31% or 70% of protein in the form of casein to young rats for a period of 44 days. At the end of the experiment they found that the kidneys of the young rats varied in size with the protein content of the diet: on the intermediate diet the kidneys were 30% larger than on the low-protein diet; on the high-protein diet they were 50% larger. In more extended studies, Smith and Moise (1927) and MacKay and MacKay (1931a) found that there is an almost proportional relationship between the protein intake and the resulting kidney weight. More recently, Konishi and Brauer (1962) have confirmed that diets high in protein will cause renal hypertrophy and Konishi (1962) has shown that diets containing 72% casein will increase the mitotic index of rat kidneys. A similar enlargement in response to high-protein diets takes place in the liver (MacKay, MacKay and Addis, 1928; Campbell and Kosterlitz, 1950), but not in the adrenals, pituitary, thyroid, testes, seminal vesicles, prostate or spleen (Leathem, 1945).

The form in which the protein is administered apparently makes

little difference apart from slight variations in the degree of hypertrophy. Wilson (1933) found that gelatin produced a more marked increase in kidney weight than did caseinogen. Baxter and Cotzias (1949) found that the intraperitoneal injection of gelatin, albumin or globulin produced reversible enlargement of the kidneys. Gelatin caused the most rapid increase in kidney size although albumin ultimately caused as much enlargement. Globulin produced less enlargement than either gelatin or albumin. Injections of casein hydrolysate did not produce similar increases in kidney size. Similarly Reid (1947) found that whole proteins could cause renal enlargement whereas diets containing mixtures of various amino acids in sufficient quantity to double the total nitrogen intake did not.

The response to unilateral nephrectomy is also affected by diet; if the animals are placed on a high-protein diet at the time of operation, the compensatory hypertrophy is greater (Moise and Smith, 1927; Allen and Mann, 1935; MacKay, Addis and MacKay, 1938; Reid, 1944; Konishi and Brauer, 1962). Konishi (1962) has also shown that diets high in protein will increase the mitotic response in the remaining kidney. Conversely, protein-free diets will depress the response to unilateral nephrectomy (Mandel, Mandel and Jacob, 1950b). The kidney weight, protein and ribonucleic acid contents of the surviving kidney are much less than with a complete diet, though the deoxyribonucleic acid content is unaffected. In other words, the increase in cell size is diminished although increase in cell number is unaffected. It is possible therefore that these two

aspects of compensatory renal hypertrophy are, to some extent, independent of one another.

Since a protein-free diet depresses the response to unilateral nephrectomy, it seems reasonable to expect that starvation will magnify this effect, for not only protein but all nutrients essential for growth are being withheld. In normal unoperated rats subjected to starvation, Kurnick (1955) has shown that there is a reduction in total RNA and protein content per kidney during the first 2 or 3 days but no change in the total DNA content per kidney or per cell, indicating that the cells are diminishing in size but not in number. The first report of the effect of starvation on compensatory renal hypertrophy is that of Sacerdotti (1896) who stated that it was inhibited. Hall and Hall (1952) also found that the increase in kidney weight following unilateral nephrectomy was almost completely suppressed if the animals were fasted. In agreement with this, Williams (1962a) has shown that mitotic activity in the kidney remaining after unilateral nephrectomy is greatly depressed in starved rats. More recently, Royce (1963) and Reiter (1965) both found that if rats were deprived of food and water after unilateral nephrectomy, the surviving kidneys did not show an increase in weight or in DNA synthesis. It may, however, be misleading to say that starvation and deprivation of water "inhibit" compensatory renal hypertrophy. It would be more accurate to say that starvation and water deprivation tend to make normal kidneys diminish in size and mitotic activity (Kurnick, 1955; Williams,

1962a). Unilateral nephrectomy tends to make the remaining kidney grow. When a rat is subjected to unilateral nephrectomy and at the same time deprived of food and water, the two effects cancel out and the remaining kidney remains the same size as before.

If the effects of high-protein diets on the kidneys are due to the increased excretory work the kidneys must do in order to remove the extra urea from the blood stream, then the addition of substantial amounts of urea to the diet should also result in kidney hypertrophy. This has not always been found to be the case. Having shown that on high-protein diets the kidneys of rats could enlarge by as much as 50%, Osborne, Mendel, Park and Winternitz (1925) found that comparable changes in kidney size could be obtained with large quantities of urea. In a more extensive paper in 1927 however, Osborne et al. fed diets containing 18-28 per cent urea to rats and compared their effect on the kidneys with those of diets containing an equivalent nitrogen intake in the form of protein. The urea-containing diets failed to cause as great a renal enlargement as the corresponding protein-containing diets. MacKay, MacKay and Addis (1931) obtained similar results. Although Wilson (1933) found a significant but small effect on kidney weight with a diet containing 10% urea, Allen and Mann (1935) found that if rats were kept on diets containing 20% urea for 8 weeks, they developed much larger kidneys than control animals on a standard diet containing no urea. MacKay, MacKay and Addis (1927b), Reid (1944) and Baxter and Cotzias (1949) on the other hand, did not

find any significant renal enlargement on urea-containing diets. It seems unlikely therefore that kidney growth is directly related to the quantity of urea the kidneys are called upon to excrete.

The other major solutes of the urine are inorganic ions. The effect of overloading the kidney with a variety of salts has therefore been extensively investigated. Osborne et al. (1927) fed diets containing a variety of salts in concentrations up to 25% for periods varying between 30 and 80 days and found that even these very high salt concentrations produced very little hypertrophy. Allen and Mann (1935) fed diets containing 15% by weight of sodium chloride to unilaterally nephrectomized rats and did not find larger kidneys than in unilaterally nephrectomized rats fed standard diets. Goss and Rankin (1960) found that rats given drinking water containing 1% sodium chloride for a period of 5 days, developed kidneys with a higher level of cell division than rats on a basal diet. When, however, the animals were unilaterally nephrectomized after 5 days on the salt diet, the mitotic response in their remaining kidneys was actually less than that observed in unilaterally nephrectomized animals not given salt in their drinking water. They explained this observation on the grounds that because the rat kidneys had already hypertrophied during the 5 days on the salt diet the growth response to unilateral nephrectomy was correspondingly reduced. Lotspeich (1965) found that substituting 1.5% ammonium chloride for the drinking water resulted in a highly significant increase in wet and dry kidney weight and in the total kidney content of nitrogen,

DNA and RNA in normal rats and in unilaterally nephrectomized rats placed on the diet at the time of operation. A diet containing an isomolar amount of sodium chloride fed to normal rats also resulted in a significant but much smaller increase in wet and dry kidney weight. Neither sodium bicarbonate nor ammonium citrate caused any significant changes in kidney weight.

Water is the one major urinary constituent which has not been greatly examined for its overloading effect. Allen and Mann (1935) and Lotspeich (1965) both noted that the animals on their sodium chloride diets drank larger quantities of water than on basal diets, but since the former found no kidney hypertrophy with the animals fed the salt diet and the latter obtained only a small response, it seems unlikely that the extra water which had to be excreted had any effect on kidney size or composition. Zakharov (1961) injected normal saline into mice (1 ml.) and rats (5 ml.) in order to flood the animals with water, thus increasing the amount to be excreted. He found that this resulted in a decrease in mitotic activity of the kidneys 1, 2 and 3 hours later.

The situation outlined in the preceding paragraphs is obviously confused. Clearly the protein content of the diet has some effect on kidney growth, but the mechanism involved is quite obscure. Since gelatin is approximately as effective as albumin or globulin, it is unlikely that the dietary protein is important as a source of essential amino acids for the formation of additional kidney tissue.

Since dietary urea is less effective than protein, the effect of protein cannot be entirely explained in terms of the consequent increase in urea output. The experiments on salt intake are equally confusing. Quite clearly they provide some evidence for the view that kidney size may be related to the need to maintain electrolyte and water balance. They do not, however, suggest that this is a factor of such importance as to dominate the process of compensatory renal hypertrophy.

4.2. The possible Role of Humoral Factors.

An alternative approach to the problem has been to postulate the existence of some specific mechanism which determines the amount of kidney tissue in an animal. Theories of this sort have been put forward from time to time, not only for kidney, but also for a wide variety of tissues and organs. (Weiss, 1952; Teir and Lahtiharju, 1961; Bullough, 1965). They have led to a search in blood serum and in homogenates and extracts of tissues for factors which stimulate mitotic activity in specific organs. In the case of the kidney, the first such experiment was performed by Sacerdotti (1896) who reported that serum from nephrectomized dogs injected into normal dogs caused an increase in kidney size after six days. Thirty years later, Cameron and Kellaway (1927) administered weekly injections of kidney homogenates to unilaterally nephrectomized guinea pigs but found that this did not affect the compensatory hypertrophy of the remaining kidney. On the other hand, at about the same time, Breuhaus and McJunkin (1932) injected

macerates of normal kidney into normal and unilaterally nephrectomized rats and reported an increase in mitotic activity in the kidneys of both groups. Much more recently, Semenova (1961) reported that kidney extracts injected intraperitoneally increased the mitotic activity and nucleic acid content of normal mouse kidneys. Extracts of liver and pancreas were ineffective.

Unfortunately, the evidence for the presence in kidney homogenates and extracts of factors which stimulate kidney growth is balanced by evidence for the presence of inhibitors. Thus Saetren (1956) spread kidney macerates in the peritoneal cavity of mice from which he had removed half of one kidney, and found that they depressed the mitotic response in the remaining kidney fragment. Macerates of liver, spleen, testis and brain were ineffective. The factor responsible for the inhibition withstood freezing, thawing, desiccation and storing at 1° for 10 days, but it was destroyed by heating to 60° for 10 minutes. Steuart (1958) injected homogenates of kidney or liver either intraperitoneally or subcutaneously into rats from which he had removed all of one kidney and half of the other. Both extracts inhibited the mitotic response produced by the operation though the kidney homogenate was the more potent. Inhibition of mitosis in kidney as a result of injecting liver homogenates has also been reported by Stich (1960), this time in normal unoperated baby rats. Stich also found that homogenates of parotid gland were inhibitory.

Some of the contradictions in experiments of the sort described in the preceding paragraphs may plausibly be attributed to technical complications. This is well illustrated by the experiments of Williams (1962b) who found that the mitotic response in unilaterally nephrectomized rats was depressed if a kidney macerate was spread over the peritoneum, but not if a macerate of liver or spleen was used instead. However, he observed that the kidney macerate diminished the animal's food intake, whereas the other two did not. In a subsequent investigation he was able to show that starvation for a corresponding period also produced an inhibition. The effect of the kidney homogenate on mitosis, therefore, might merely be an indirect result of the reduction in food intake which it caused, and therefore of no fundamental significance.

In a similar series of experiments, however, Roels (1965) found, as Williams (1962b) had done, that intraperitoneal injections of kidney homogenate into unilaterally nephrectomized rats caused an inhibition of mitosis 47 hours after nephrectomy. Since however the body weights of the animals were not significantly different from controls, Roels did not agree with Williams (1962b) that these results were due to undernourishment. He concluded instead that they indicated the existence of a hormone produced by the renal cells and controlling mitotic activity in the nephron; unilateral nephrectomy might reasonably be expected to cause a fall in the concentration of this "hormone" and a consequent rise in mitosis. This idea was first put forward as a general theory of

compensatory hypertrophy in damaged tissues by Bullough (1965).

Goss (1963a) gave injections of various tissue homogenates to unilaterally nephrectomized rats about 30 hours after operation and examined their effect on the mitotic activity 18 hours later. Intraperitoneal injection of homogenates of fresh, cooked or frozen kidneys or of suspensions of trypsin-dissociated kidney, all reduced the mitotic activity by about half. Intraperitoneal injections of fresh liver, testis, spleen and blood homogenates, however, inhibited renal mitosis just as effectively, and so for that matter did egg albumin. Subcutaneous injection of frozen kidney homogenate or intraperitoneal injection of saline, Hanks balanced salt solution, fresh plasma, plasma of unilaterally nephrectomized rats or fresh egg yolk however, had no effect on the mitotic index. Obviously these results provide no evidence of tissue-specific growth regulating agents.

All the experiments described above are open to the general criticism that they involve the introduction into the recipient animal of large quantities of kidney protein (and other constituents) which might well be expected to affect its metabolism, quite regardless of the content of any supposed hormone. This difficulty is avoided in the alternative type of experiment in which the test animals receive injections of serum from donor animals. For example, Williams (1962b) injected serum in this way into rats which had been unilaterally nephrectomized. He found that no matter whether the serum came from sham operated rats or from rats which had themselves

been nephrectomized, it had no effect on compensatory renal hypertrophy in the recipients. Goss (1963a), in a similar series of experiments, obtained similar results. Both he and Williams gave their animals only one injection. Lowenstein and Stern (1963), who gave two injections a day for four days, reported that serum from unilaterally nephrectomized rats increased DNA synthesis (measured with tritiated thymidine) in the kidneys (but not the livers) of normal rats.

The problem of transferring sufficient of the supposed hormone from donor to recipient can, theoretically, be overcome by using parabiotic rats. Steuart (1958) combined rats in threes to make up parabiotic "triplets". He then carried out bilateral nephrectomies on each of the two outer animals in such a preparation and reported that this caused a burst of mitosis in the central animal.

A special case of parabiosis is represented by the relationship of mother and foetus. Goss (1963b) found that when one maternal kidney was removed on the nineteenth day of gestation, there was no evidence of compensatory renal hyperplasia in the foetal kidneys two days later, although the surviving maternal kidney showed a three-fold increase in mitotic activity. Rollason (1961) studied the effect or removing both kidneys from rats pregnant for eighteen and a half days. One day later the ratio of foetal kidney weight to body weight was less than usual but this returned to normal on the second and third days. The mitotic

activity of the kidneys was unchanged. These negative results may be due to the fact that the foetal kidneys were already growing maximally. Alternatively the placenta may exclude from the foetus any growth-controlling factors circulating in the maternal blood stream.

Apart from the question of kidney specific growth factors there is also the possibility that the kidney may be subject to the action of more general growth-promoting mechanisms. Paschkis, Goddard, Cantarow and Adibi (1959) found that rats subjected to combined unilateral nephrectomy and partial hepatectomy developed kidneys about 50% heavier than those in unilaterally nephrectomized controls. However Simpson (1961b) found that in normal rats partial hepatectomy did not increase DNA synthesis in the kidneys as measured by ^{32}P incorporation. These two results are rather at variance and it is difficult to form any definite conclusion.

Theoretically it should be possible to test for the presence of growth promoting or inhibiting factors in tissue extracts, or in plasma, by using kidney cells growing in vitro in tissue culture. By this technique, Ogawa and Nowinski (1958) found that serum from unilaterally nephrectomized rats added to the tissue cultures gave mitotic activities twice as high as normal serum. The growth promoting factor was organ specific since serum incubated with cells cultured from the bladder, anterior pituitary or pancreas of the rat, did not affect the mitotic activities of these tissues. The factor was not, however, species specific, since the serum increased three

fold the mitotic activity of puppy kidney cultures. It was destroyed by boiling.

To sum up: the results described in this section are too confused and contradictory to allow any firm conclusions to be drawn; obviously however, the case for some sort of humoral control of compensatory renal hypertrophy is strong enough to warrant further investigation.

4.3. Endocrine Effects on Compensatory Renal Hypertrophy.

In considering the possibility that compensatory renal hypertrophy is due to a functional overload or to the operation of some humoral factor, it is essential to bear in mind the well-known effects of endocrine glands on kidney growth and function.

One of the chief functions of the kidney is the maintenance of electrolyte and water balance. The volume of water excreted by the kidneys is determined by the concentration of antidiuretic hormone (ADH) in the blood. When the plasma osmotic pressure increases above normal, the neurohypophysis is stimulated to release more ADH. This increases water reabsorption in the distal kidney tubules so that the rate of urine secretion falls. Conversely when the plasma osmotic pressure falls below normal, less ADH is released, water reabsorption diminishes and the rate of urine secretion rises. Since the antidiuretic hormone has such a direct influence on kidney function, it seems possible it might equally affect kidney growth. This has not been experimentally investigated, nor is it known if unilateral nephrectomy affects the rate at which the hormone is

secreted by the neurohypophysis.

There is however no doubt that the pituitary, as a whole, does influence kidney growth. After hypophysectomy, the size of the kidney decreases (Smith, 1930; Selye, 1941; Levin, 1944; Fontaine, 1947). Goss and Rankin (1960) have reported that hypophysectomy produces a substantial drop in mitotic activity in the kidneys. On the other hand, McCreight and Sulkin (1962) have found that mitotic activity in normal kidneys is very low in any case and that hypophysectomy has correspondingly little effect. The atrophy of the kidney after hypophysectomy cannot be prevented by feeding a high protein diet (Leathem, 1945), and even when hypophysectomised rats which have lost body weight and kidney weight are forcibly fed quantities of food sufficient to prevent the loss in body weight, the relative weight of the kidney remains depressed (Levin, 1944).

Growth of the kidney remaining after unilateral nephrectomy is also dependent on the pituitary (Astarabadi, 1962a). Whether hypophysectomy completely abolishes compensatory renal hypertrophy or merely depresses it, is the subject of conflicting reports. The difficulty here is that the common criterion of hypertrophy is increase in the weight of the remaining kidney. This is a slow process which continues for 30 days or more (Section 1.2.1). During such a prolonged period the body weight of the animal may change. Rats in particular may increase in weight by up to 50% (Astarabadi and Essex, 1953). The weight increase in the surviving

kidney in such circumstances, therefore, is partly attributable to compensatory hypertrophy and partly to the general growth of the animal. In otherwise normal animals, hypophysectomy affects the mass of the kidney in two ways: it causes a regression in kidney size relative to body weight; and it also results in a cessation of growth of the animal (Rolf and White, 1953). Both these effects must be taken into account in assessing the effect of hypophysectomy on compensatory renal hypertrophy. When this is done, it is apparent that hypophysectomy does not abolish the hypertrophy (Astarabadi and Essex, 1953; Rolf and White, 1953). This is in agreement with the observation that after unilateral nephrectomy of a hypophysectomised rat, the surviving kidney does show the normal type of mitotic response though to a diminished degree (Goss and Rankin, 1960; McCreight and Sulkin, 1962). On the other hand, the kidney does influence the pituitary, for Wrete (1946) has shown that unilateral nephrectomy in mice causes pituitary enlargement.

The pituitary may influence kidney growth either directly or via the other endocrines. Hay (1946) has shown that a highly purified thyrotropic preparation from the anterior pituitary increased kidney size in normal and hypophysectomized rats. MacKay and MacKay (1931b) have shown that administration of a diet containing ~~dessicated~~ DESICCATED thyroid to male rats was followed by a marked increase in kidney weight, greater than could be accounted for by the corresponding increase in protein intake. Thyroxine itself

increases the weight of the kidneys (Herring, 1917; Walter and Addis, 1939; Selye, Stone, Nielsen and Leblond, 1945), their mitotic index (Pisi and Cavalli, 1955) and their content of RNA and protein (Mandel and Revel, 1958). Conversely, thyroidectomy results in a reduction of kidney weight in otherwise normal animals (MacKay and MacKay, 1931b; Walter and Addis, 1939), but does not prevent compensatory renal hypertrophy taking place after unilateral nephrectomy (Zeckwer, 1946).

Adrenocorticotrophic hormone on the other hand, has little or no effect. It causes no change in kidney weight or histology (Simpson, Li and Evans, 1946). It does not reverse the reduction in kidney weight brought about by hypophysectomy (Astarabadi, 1962b) and it fails to restore normal compensatory renal hypertrophy in hypophysectomised animals (Astarabadi, 1963a; McCreight and Reiter, 1965). In agreement with these negative findings, adrenalectomy does not greatly affect the level of mitotic activity in the intact kidney (Williams, 1962a; Goss, 1965) although it distorts the normal diurnal rhythm (Williams, 1962a). In contrast to these findings however, Reiter and McCreight (1965b) found that adrenalectomy of otherwise intact rats significantly increased the uptake of tritiated thymidine in both cortex and medulla. This seems to indicate an increase in DNA synthesis following adrenalectomy which is difficult to reconcile with the lack of mitotic response.

There has been some disagreement on the effects of adrenalectomy on compensatory renal hypertrophy. Goss and Rankin (1960) found

that it abolished the mitotic response of the remaining kidney 48 hours after unilateral nephrectomy. Goss (1965) subsequently showed that the normal response was restored if the rats were given deoxycorticosterone. Williams (1962a), on the other hand, found that the mitotic response was only slightly depressed and Astarabadi (1963a) found that adrenalectomy made virtually no difference to the increase in weight of the surviving kidney in the two weeks following unilateral nephrectomy.

These discordant results have been explained by Goss (1965) as being due to variations in salt intake. Whereas Williams (1962a) and Astarabadi (1963a) added salt to the drinking water of their animals post-operatively, Goss and Rankin (1960) did not. Goss (1965) has since shown that adrenalectomy considerably depresses the mitotic response after unilateral nephrectomy in animals given fresh water to drink but not in animals given 0.9% sodium chloride. In agreement with this, Reiter and McCreight (1965b) found that if rats were given saline drinking water, adrenalectomy had very little effect on DNA synthesis in the surviving kidney after unilateral nephrectomy. It seems reasonable therefore to assume that an adequate sodium level is in some way a pre-requisite for renal hypertrophy; and that the importance of the adrenals in compensatory renal hypertrophy is that the mineralocorticoids they produce promote sodium retention. There is a certain amount of supporting evidence for this. Deoxycorticosterone given to normal rats causes an increase in kidney weight and mitotic activity

(Ludden, Kreuger and Wright, 1941; Selye, 1941; Goss, 1965), and aldosterone increases their RNA content (Castles and Williamson, 1965).

Several of the other steroid hormones also exert a growth-promoting effect on the kidney. Although cortisone acetate, in contrast to deoxycorticosterone and aldosterone, has been shown to completely prevent compensatory renal hypertrophy (Goss and Rankin, 1960), testosterone administered to normal rats significantly increases kidney weight (Ludden, Kreuger and Wright, 1941; Selye, 1941; Kochakian and Stettner, 1948) and response to unilateral nephrectomy (MacKay, 1940; Lattimer, 1942; Berech and Curtis, 1964). Kassenaar, Kouwenhoven and Querido (1962) found a decrease in kidney weight and total renal content of RNA in mice as a result of castration; treatment with testosterone reversed these changes. Leathem (1948) has also reported that castration decreased kidney weight but MacKay (1940) found it had no effect on either kidney weight or the degree of compensatory renal hypertrophy after unilateral nephrectomy. Progesterone (Selye, 1941) and oestrogen (Ludden et al. 1941; Selye, 1941) both have growth promoting effects on the kidney. Schaffenburg and McCullagh (1953) however, found that oestrogens in small 'physiological' doses had no effect on compensatory renal hypertrophy after unilateral nephrectomy, whereas larger doses caused a significant inhibition of this hypertrophy.

Growth hormone produces an increase in kidney weight in normal

rats (Kochakian and Stettner, 1948). It also stimulates compensatory renal hypertrophy in unilaterally nephrectomized hypophysectomized rats (Astarabadi, 1963b). The decrease in kidney size produced by hypophysectomy in intact rats is, however, less completely reversed by growth hormone than by crude pituitary extracts (Astarabadi, 1962b). Growth hormone may therefore be important for kidney growth, but it can scarcely be considered a specific renotropic hormone.

The possible role of the endocrines in compensatory renal hypertrophy could therefore be summed up somewhat as follows. The pituitary, as a whole, obviously has a considerable influence on kidney size. Whether this is exerted through the action of the antidiuretic hormone on kidney function is uncertain. The available evidence however indicates that some degree of compensatory renal hypertrophy can take place even in hypophysectomized rats. Clearly therefore, the mechanism of compensatory renal hypertrophy does not necessarily involve the pituitary. So far as other hormones, steroid or otherwise, are concerned, it seems clear that while again they may exercise a greater or lesser influence on kidney size and on the speed and extent of compensatory renal hypertrophy, they have not been shown to perform any essential role in compensatory renal hypertrophy. This is perhaps hardly surprising. All the hormones discussed above (with the exception of the antidiuretic hormone) exert a general action on all or most tissues of the organism. It seems unlikely therefore that any of them should

exert a specific control over the kidney.

4.4. Miscellaneous theories.

Most of the workers who have investigated the problem of compensatory renal hypertrophy have assumed that it is a response either to a functional overload or to some sort of humoral mechanism which predetermines the amount of kidney tissue in the organism. From time to time however, radically different theories have been put forward. For example, Arataki (1926) suggested that after unilateral nephrectomy the surviving kidney had to perform more work, that this resulted in an increased blood supply and that this in turn was the immediate stimulus to hypertrophy. There is at least some evidence that this is not so. Idbohrn and Muren (1956) found that if one ureter is ligated in a rabbit the corresponding kidney underwent a marked increase in weight at a time when its blood supply was actually diminished by 60%. In this case at least there seems to be little relationship between blood supply and growth. If, however, compensatory renal hypertrophy was found to be associated with increased renal blood flow it would not necessarily follow that the one caused the other. In any case it is not clear in what way unilateral nephrectomy might be expected to increase blood flow to the surviving kidney.

Goss and Rankin (1960) have sought a possible relation between compensatory renal hypertrophy and the renal function of regulating blood pressure. Since, however, it has never been shown that blood pressure rises after unilateral nephrectomy, it seems unlikely that

this mechanism exerts any control over the resulting kidney growth.

5. The situation in 1963.

Although the process of compensatory renal hypertrophy has been investigated for more than a century, it is clear from the work summarised above, that the subject is still confused. This is partly because there have been so many conflicting reports and partly because there is still no clear indication of the mechanism controlling the process. One of the major problems of the work so far is that much of it is now fairly old and based on methods of measuring growth of the kidney which cannot now be regarded as quantitative. For this reason it seemed necessary to start a fresh study of the problem from the beginning.

Section 2

MATERIALS AND METHODS

M A T E R I A L S A N D M E T H O D S.

1. General.

1.1. Animals.

Adult albino rats and mice from the departmental colony were used in all experiments. The rats were males unless otherwise stated. Their body weights were in the range 120-330 g. They were housed individually under thermostatic conditions (26°) and were normally fed on stock diet (Table 2). In dietary experiments the rats were provided with their diets in individual feeding dishes.

1.2. Isotopes.

Tritiated adenine labelled at positions 2 and 8 in the purine ring (Code number TRA.23) of specific activity 2,360 mc/mM and tritiated orotic acid labelled at position 5 of the pyrimidine ring (Code number TRA.84) of specific activity 2,300 mc/mM were obtained from the Radiochemical Centre, Amersham. Both isotopes were stored at -10° until required for use. Amounts varying between 1 μc per 2 g. and 2 μc per g. body weight were injected intraperitoneally.

1.3. Diets.

By trial and error it was found that 12 to 15 g. of stock diet (Diet 41, Bruce and Parkes, 1949) per day was an adequate food intake for rats of 150-200 g. body weight. The composition of diet 41 is given in Table 2.

In dietary experiments the animals were fed each morning at

10.00 a.m. after an initial 17 hour fast. All animals were offered a fixed intake of diet. When synthetic diets were used, the calorie intake was controlled in order to provide about 1,450 calories per square metre body surface area per day. This is necessary for nitrogen balance to be maintained (Munro and Naismith, 1953). The body surface area was computed from the formula $S = 12.54 \times W^{0.60}$ sq. cm. where S is the body surface area and W is the body weight in grams (Lee, 1929). The composition of the semi-synthetic diets used and the amounts offered to the animals are shown in Tables 3-6.

1.4. Surgical procedures.

All operations were performed between 9.00 a.m. and 12.00 noon.

(a) Unilateral nephrectomy.

Right unilateral nephrectomy was performed under ether anaesthesia through a midline abdominal incision. The kidney was decapsulated and the renal pedicle ligated with linen thread at a point about 3 mm. from the kidney, which was then excised. The wound was closed in a single layer with interrupted sutures. The kidney was blotted free of blood and urine on filter paper moistened with isotonic saline and weighed fresh on a torsion balance. The kidneys were frozen in a mixture of alcohol and solid carbon dioxide and stored at -70°. Sham operations were performed by making a similar incision, locating the right kidney, handling it for a length of time equivalent to that required for excision and closing the wound in the manner described above.

Table 2.

Calculated analysis of diet 41 (Bruce & Parkes, 1949)

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

Table 3.

Composition of semi-synthetic diets.

	Protein-free	High-protein
Casein (g.)	-	240
Margarine (g.)	42	42
Glucose (g.)	569	449
Potato starch (g.)	189	69
V.M.R.* (g.)	100	100

* See Table 4.

A 150 g. animal was offered 10 g. per day of one of these diets. The physiological calorie equivalents are:

Protein	4 calories per gram.
Carbohydrate	4 calories per gram.
Fat	9 calories per gram.
V.M.R.	3.5 calories per gram.

The diets as made up therefore had a calorie equivalent of 4.18 calories per gram of diet.

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.

* See Table 5.

† See Table 6.

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

Table 5.

Composition of salt mixture "446".

NaCl	243.2 g.
Potassium citrate	533.0 g.
KH_2PO_4	174.0 g.
$CaHPO_4$	800.0 g.
$CaCO_3$	368.0 g.
Ferric citrate. $3H_2O$	36.0 g.
$CuSO_4.5H_2O$	0.4 g.
$CoCl_2.6H_2O$	0.2 g.
$K_2Al_2(SO_4)_4.24H_2O$	0.2 g.
NaF	0.002 g.
$MgCO_3$	92.0 g.
$MnSO_4$	2.8 g.
KI	0.1 g.
$ZnCO_3$	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-amino benzoic acid	500 mg.
Inositol	1.0 g.
Choline chloride	10.0 g.
Folic acid	trace.
Potato starch	to 500 g.

(b) Liver biopsy.

A midline abdominal incision was made from about 1 cm. above the xiphoid process to about 2 cm. below it. Using gentle pressure on the lower part of the thorax and upper abdomen, the median and left lateral lobes of the liver were delivered through the incision. A loop of linen thread was placed over the left radicle of the median lobe and tightened. This radicle was then excised. The abdominal wound was closed in a single layer with interrupted sutures.

1.5. Estimation of tissue dry weight.

On removal from the animal the tissue was minced finely with scissors on a weighed watch-glass which was then placed in an oven at 70° for 72 hours. The watch-glass was then placed in a desiccator over phosphorus pentoxide and weighed on a torsion balance at 24 hour intervals until a constant weight was obtained on two successive weighings.

1.6. Histological methods.

Kidneys removed from the animals were cut transversely into three portions which were fixed, dehydrated, cleared and embedded in paraffin wax according to the schedule given in Table 7. Initially Bouin's solution was used for fixation of the kidneys but the kidney tubules in the resulting stained sections were found to be collapsed, with no distinct lumen. The use of 10% formol saline was found to prevent this. The tissues were sectioned (7 μ) through the mid-transverse region and stained with haemalum and eosin according to

Table 7.

Schedule for fixing, dehydrating, clearing and embedding tissue.

Bouin's solution	18-24 hours
or	
10% formol saline	
Wash in water	½ hour
50% alcohol	12 hours
70% alcohol	12 hours
96% alcohol	12 hours
Absolute alcohol	12-24 hours (change once)
Chloroform	Overnight
Xylol till clear	Usually ½ hour
Paraffin	6 hours (change once)
Block in fresh paraffin	

Table 8.

Schedule for staining.

Haemalum and eosin :	Xylol	6 min. (change once)
	Absolute alcohol	6 min. (change once)
	Methylated spirits	3 min.
	Water	2 min.
	Iodine	2 min.
	Hypo	2 min.
	Water	2 min.
	Haemalum	2-2½ min.
	Water	Rinse
	Acid alcohol	until decolourisation stops
	Water	2 min.
	Scott's tap water substitute	until blue
	Water	2 min.
	Eosin	1 min.
	Water	Rinse
	Absolute alcohol	1 min.
	Xylol	2 min.

The slides were mounted in D.P.X.

the schedule given in Table 8. The sections were examined under oil immersion and the tubule mitoses in 600 fields (about 40,000 cells) in the cortex of the kidney were counted. The number of nuclei in each tenth field examined was also counted and the mean value calculated. From these measurements, the number of mitoses per 10,000 nuclei was calculated.

2. Chemical estimations.

1. Extraction of RNA and DNA.

The method was modified from that of Schmidt and Thannhauser (1945) (Munro and Fleck, 1966). The reagents used were as follows :

A. 0.6N-perchloric acid (PCA)

B. 0.2N-perchloric acid

C. 0.3N-potassium hydroxide (KOH)

The tissue was homogenised in 49 volumes of ice-cold glass-distilled water in a Nelco Blendor at 0° for 2 minutes. A 5 ml. aliquot of this homogenate (containing 100 mg. wet weight tissue) was pipetted into a centrifuge tube and 2.5 ml. of ice-cold 0.6N-PCA added. After thorough mixing, the mixture was allowed to stand at 0° for 10 minutes. The precipitate of protein and nucleic acids was then separated by centrifugation at 1,000g for 10 minutes and washed twice with ice-cold 0.2N-PCA. The supernatant and washings were discarded. The excess PCA was carefully drained off, the sides of the tubes wiped and 4 ml. of 0.3N-KOH added. Digestion was then carried out in a shaking water bath at 37° for 1 hour.

At the end of the incubation, the samples were chilled in ice and the DNA and protein precipitated by the addition of 5 ml. of 0.6N-PCA. The precipitate was collected by centrifugation and washed twice with 0.2N-PCA. The supernatant and washings were combined, made up to 50 ml. and a final concentration of 0.1N-PCA. This was the RNA fraction. The precipitate was dissolved in 5 ml. 0.3N-KOH and made up to 25 ml. and a final concentration of 0.1N-KOH. This was the DNA fraction. A flow sheet summarising the extraction procedure is given in Figure 2.

2.2. Estimation of RNA in the extract.

The RNA content of the perchloric acid extract was estimated on the basis that an extinction of 1.000 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. Estimation of DNA in the extract.

The DNA content of the extract was estimated by the method of Ceriotti (1952; 1955). The reagents used were as follows:

A. Indole. 0.04% (w/v) in distilled water.

B. Concentrated hydrochloric acid (Analar S.G. 1.19).

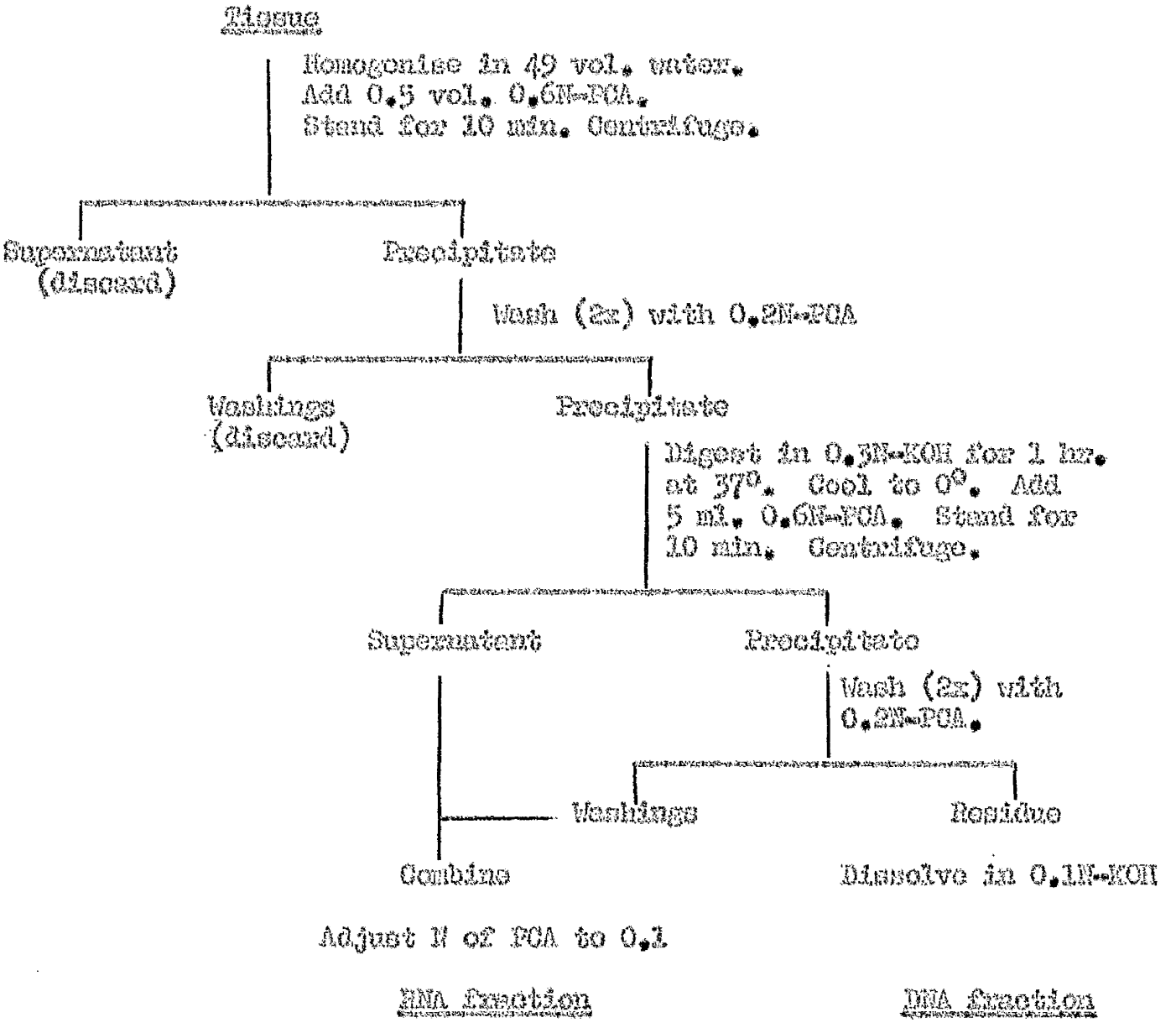
C. Chloroform (reagent grade).

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. DNA were dissolved in distilled water with a drop

Figure 2

PROCEDURE FOR THE SEPARATION OF RNA AND DNA

All operations were performed at 0° unless otherwise stated.



of N-NaOH to help solution, the final volume being 50 ml. A 1 ml. aliquot of this solution was diluted with 0.5N-PCA, heated to 70° for 20 minutes to redissolve any precipitated DNA, and made up to 50 ml. The amount of deoxyribonucleic acid phosphorus (DNAP) in this standard was estimated by the method of Griswold, Hummöller and McIntyre (1951) (Section 2.2.9.).

2 ml. of the DNA solution, 1 ml. indole reagent 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 three times with 4 ml. portions of chloroform, shaking for about 45 seconds after each chloroform addition. After the third extraction, the tubes were centrifuged at 200g for 5 minutes to aid separation of the aqueous and chloroform layers. The extinction of the aqueous layer was read at 490 mμ in a Beckman DB spectrophotometer. Blanks consisting of 2 ml. distilled water and standards containing 2 ml. of the standard DNA solution were also carried through this procedure.

2.4. Extraction of RNA labelled with [^{3}H] adenine.

In some experiments the tritium content of RNA was estimated following extraction by the method described in section 2.2.1. This

method is more quantitative than the phenol extraction technique normally used for the extraction of labelled RNA (section 2.5.). Since, however, the tritium content of the RNA was never very high, it was essential to modify the method in such a way as to give the RNA fraction in a small volume. The reagents used were as follows:

A. 0.6N-PCA.

B. 0.2N-PCA.

C. 0.3N-KOH.

D. 60% (v/v) PCA.

E. 7N-KOH.

The kidneys were homogenised, extracted with 0.6N-PCA and washed with 0.2N-PCA as above (Section 2.2.1.). The acid-soluble fractions were combined and retained for isotope determination (Section 2.7.). The residue was incubated at 37° for 1 hour with 0.3N-KOH. The minimum volume of KOH required was estimated from two separate experiments in which samples extracted from the one kidney homogenate were incubated with varying amounts of 0.3N-KOH. As Table 9 and Figure 3 show, incubation with 4, 3 or 2 ml. KOH gave the same results, but incubation with 1 ml. KOH or less, resulted in incomplete digestion of the RNA. In order to have a small margin of safety, it was decided that 2.5 ml. KOH should be used in subsequent incubations in radioactive experiments of this sort. Following incubation, the samples were chilled in ice and 60% (v/v) PCA added to a final concentration of 0.2-0.3N. The

Table 9.

The effect of the amount of alkali in the alkaline digestion on the recovery of RNA.

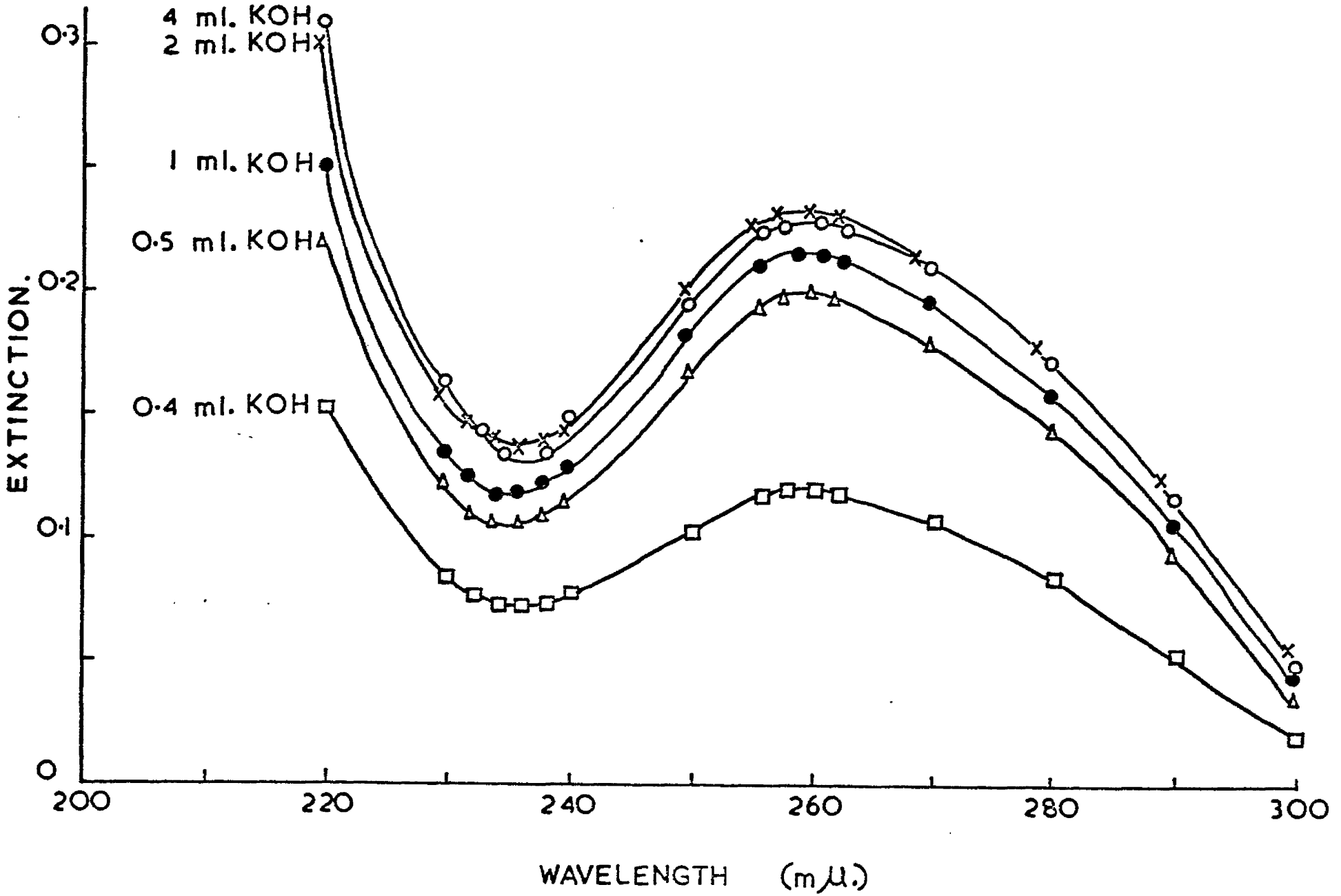
Volume Of 0.3N-KOH Used In Digestion. (ml.)	RNAP Recovered In Digest (µg/kidney)
4.0	315
3.0	315
2.0	310
1.0	302
0.5	241
0.4	150

Values are means of two determinations.

Figure 3

The effect of the amount of alkali (0.3N-KOH) used in the alkaline digestion on the recovery of RNA.

FIGURE 3.


4 ml. KOH
2 ml. KOH
1 ml. KOH
0·5 ml. KOH
0·4 ml. KOH
EXTINCTION.
0·3
0·2
0·1
0
200
220
240
260
280
300
WAVELENGTH (mμ.)

precipitate of DNA and protein was removed by centrifugation. Since PCA interferes with the estimation of radioactivity in a liquid scintillation counter (Section 2.7.), the PCA was removed from the supernatant (RNA fraction) by neutralising it with 7N-KOH. The mixture was then left at 0^{o} overnight to precipitate the $KClO_4$. After centrifugation, the supernatant was transferred to a 10 ml. measuring cylinder and the volume noted. A 0.5 ml. aliquot was made up to 10 ml. with distilled water and the extinction at 260mμ read in a Beckman DB spectrophotometer. From this, the amount of RNAP present was calculated as in section 2.2.2. An 0.8 ml. aliquot was taken for tritium assay as described in section 2.7.

2.5. Estimation of protein.

Estimation of protein was carried out by the method of Lowry, Rosebrough, Farr and Randall (1951). The reagents used were as follows:

A. 2% (w/v) sodium carbonate in 0.1N-sodium hydroxide.

B. 0.5% (w/v) cupric sulphate ($CuSO_4.5H_2O$) in 1% (w/v) 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) diluted with distilled water so that it was 1N with respect to acid.

The tissue was homogenised in 49 volumes ice-cold distilled water in a Nelco Blendor. 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 SP 500 spectrophotometer. The assay was calibrated using a standard aqueous solution of bovine serum albumin.

2.6. Extraction of protein for protein nitrogen estimation.

The reagents used were as follows:

A. 21% (w/v) trichloracetic acid (TCA).

B. 7% (w/v) TCA.

C. 0.3N-KOH.

An aqueous homogenate of kidney was prepared in a Potter homogeniser such that 5 ml. of homogenate contained 100 mg. wet weight kidney. 2.5 ml. 21% TCA were added to 5 ml. of the homogenate in a centrifuge tube. The solution was mixed and allowed to stand for 10 minutes at 0^{o}. The precipitate of protein and nucleic acid was separated by centrifugation and washed twice with 7% TCA. It was then dissolved in 4 ml. 0.3N-KOH and made up to 8 ml. with water. The mixture was centrifuged at 1,000g for 5

minutes and 2 ml. of the supernatant taken for nitrogen estimation by the micro - Kjeldahl method.

2.7. Estimation of protein nitrogen.

The reagents used were as follows:

A. Concentrated sulphuric acid.

B. Mercury catalyst. (4 g. HgO in 100 ml. 4N-sulphuric acid).

C. Potassium sulphate.

D. Zinc dust.

E. 40% (w/v) sodium hydroxide.

F. 0.01N-sulphuric acid.

G. 0.01N-sodium hydroxide.

H. De Wesselow's indicator.

I. Standard ammonium sulphate solution (10 ml. = 1 mg. N, B. D. H.).

Material containing 0.5-2.0 mg. nitrogen was placed in a digestion flask and 1.5 ml. concentrated sulphuric acid, 1 ml. mercury catalyst solution and 1.2 g. potassium sulphate added. The sides of the flask were washed down with a little distilled water. The flask was transferred to a digestion rack, gently heated to boil off the water and then digested over maximum heat for ½ hour. Simultaneously, duplicate blanks were treated in the same way. After digestion, the sides of the flask were cooled with water and the contents transferred quantitatively to the Markham

apparatus (Markham, 1942). Zinc dust (0.2 g.) was added to the apparatus together with a further two washings from the digestion flask. 10 ml. 40% NaOH was then slowly added to the apparatus. Steam distillation was carried out for 2 minutes, the ammonia being trapped in 10 ml. 0.01N-H_2SO_4 and titrated with 0.01N-NaOH using De Wesselow's indicator (end-point is green colour after purple to colourless change). A standard solution of ammonium sulphate was treated in the same way. From the results, the protein nitrogen was obtained by subtraction of the nitrogen content of the nucleic acids present. Thus protein nitrogen = total nitrogen - (RNAP + DNAP) x 1.69.

2.8. Extraction of phospholipid.

The method used was based on that of Folch, Lees and Sloane-Stanley (1957). The reagents used were as follows:

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 Blendor. 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 two extracts were combined, shaken with 4 ml. 0.73% sodium chloride and centrifuged at 1,000g 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 transferred to the measuring cylinder and made up to the mark with methanol.

2.9. Estimation of phosphorus.

Analysis of the lipid extracts for phosphorus was performed according to the following modification of the method of Griswold et al. (1951). The reagents used were as follows:

A. 10N-sulphuric acid.

B. 4N-PCA.

C. Potassium dihydrogen phosphate standard. 2.193 g. KH_2PO_4 were dissolved in 500 ml. distilled water. 1 ml. of this solution diluted to 500 ml.

with distilled water gave a final concentration of 2 μg^P/ml.

D. Reducing agent. 13.6 g. sodium metabisulphite, 1 g. sodium sulphite ($Na_2SO_3.7H_2O$) and 0.25 g. 2-naphthol-1 amino-4 sulphonic acid (B. D. H.) in 250 ml. distilled water.

E. Aqueous 2.5% (w/v) ammonium molybdate.

Aliquots of 1 ml. of the pooled lipid extracts were evaporated to dryness in graduated test tubes in a sand bath at 100°. To the residue, 0.5 ml. 10N-sulphuric acid and 0.5 ml. 4N-PCA were added and the mixture digested until it was clear. The same procedure was carried out using 1 ml. of water (blank) and 1 ml. of diluted standard solution. The tubes were cooled and the solutions diluted to approximately 3 ml. with distilled water. To each tube 0.5 ml. reducing agent and 0.5 ml. 2.5% (w/v) ammonium molybdate were added, with careful mixing after each addition. The volumes were made up to 5 ml. with distilled water and the tubes heated in a boiling water bath for 10 minutes. The intensity of the colour was read in a Unicam SP 500 spectrophotometer at 820 mμ.

3. Enzyme analysis.

1. DNA deoxynucleotidyltransferase (2.7.7.7.).

The number in parenthesis after each enzyme is the Enzyme Commission number. The reagents used were as follows:

A. 0.01 M tris-(hydroxy-methyl)-amino-methane buffer (tris buffer), pH 7.5.

B. Tris-HCl buffer-salt mixture. This mixture contained 50 µl. 0.4 M-tris-HCl buffer, pH 7.5, 20 µl. 1 M-KCl, 10 µl. 0.02 M-diaminoethanetetra-acetic acid, 5 µl. 0.5 M-$MgCl_2$.

C. DNA. Thermally denatured Landschutz ascites-cell DNA, 2 mg./ml. in water.

D. Triphosphate mixture. A mixture containing 10 µl. of each of the following triphosphates: dATP, dGTP, dCTP, (α-^{32}P)-dTTP. Each of the triphosphate solutions contained 10 µmoles per ml. The specific activity of the dTTP was 1.45 c/min./µmole.

E. 5% (w/v) TCA.

F. 95% (v/v) alcohol.

G. Diethyl ether.

Rat kidneys were homogenised in 4 volumes of 0.01 M tris-HCl buffer, pH 7.5 in a Potter homogeniser. The homogenate was centrifuged at 18,000g for 1 hour. The supernatant was decanted and assayed for enzyme activity as described by Keir (1962).

85 µl. of tris-HCl buffer-salt mixture (reagent B), 50 µl. DNA (reagent C), 40 µl. triphosphate mixture (reagent D) and 20 µl.

DNA deoxynucleotidyltransferase fraction were added to a 3 ml. round-bottomed centrifuge tube. The volume was made up to 0.25 ml. with distilled water. The tubes were sealed with "parafilm" to prevent evaporation and incubated in a shaking water bath at 37° for 3 hours. After incubation, the reaction was stopped by freezing the tubes in solid CO_2-ethanol mixture. The frozen incubation mixtures were thawed and 50 µl. portions pipetted on to numbered discs of Whatman No. 1 filter paper, 2.5 cm. in diameter. The discs were dropped into a beaker containing ice-cold 5% TCA (15 ml. per disc) and allowed to stand for 15 minutes, the beaker being swirled gently at intervals. The TCA was decanted, replaced with an equal volume of fresh TCA and allowed to stand as before. This procedure was repeated twice more. In the same manner, the discs were washed twice with cold 95% alcohol and once with ether and dried on stainless steel planchettes. The ~~tritium~~ ^{32}P of the DNA precipitated on the discs was assayed in a Nuclear Chicago gas flow counter.

3.2. Deoxyribonuclease 1. (3.1.4.5.) (DNase 1).

The enzyme extract was prepared as for the DNA deoxynucleotidyltransferase assay (Section 2.3.1.). The reagents used for the DNase 1 assay were as follows:

A. Tris-HCl buffer-salt mixture. This mixture contained 250 µl. 0.4 M-tris-HCl buffer, pH 7.5, 100 µl. 1 M-KCl, 25 µl. 0.5 M-$MgCl_2$.

B. DNA. Thermally denatured Landschutz ascites-cell DNA, 2 mg./ml. in water.

C. Bovine serum albumin. 2 mg./ml. in water.

D. 2.1N-PCA.

375 µl. of tris-HCl buffer-salt mixture, 300 µl. DNA and 100 µl. DNase 1 fraction were added to a 3 ml. round-bottomed centrifuge tube. The volume was made up to 1.25 ml. and the tubes were incubated at 37° for 3 hours. Controls without enzyme were also incubated. After incubation, the reaction was terminated by freezing the assay tubes in a solid CO_2-ethanol mixture. The tubes were thawed and 0.25 ml. of a solution of bovine serum albumin was added to each tube as a co-precipitant, followed by 1.5 ml. of 2.1N-PCA. After shaking vigorously to ensure even distribution of acid, the tubes were allowed to stand for 10 minutes, before being centrifuged at 700g for 15 minutes to sediment precipitated DNA and protein. The supernatant fractions were decanted into fresh tubes and their extinctions at 260 mµ measured in a Unicam SP 500 spectrophotometer.

3.3. Deoxyribonuclease II (3.1.4.6.)(DNase II).

The enzyme extracts were prepared as for the DNA deoxynucleotidyl-transferase assay (Section 2.3.1.). The reagents used for the DNase II assay were as follows:

A. 0.5 M-sodium acetate buffer, pH 4.5.

B. 1.5 M-KCl.

C. DNA. Landschutz ascites-cell DNA, 1 mg./ml. in water.

D. Bovine serum albumin (2 mg./ml. in water).

E. 1N-PCA.

30 µl. acetate buffer, pH 4.5, 30 µl. KCl, 150 µl. DNA and 100 µl. DNase II fraction were added to a 3 ml. round-bottomed centrifuge tube. The volume was made up to 0.35 ml. with water and the tubes were incubated at 37° for 1 hour. The reaction was stopped by freezing the assay tubes in solid CO_2-ethanol mixture. The tubes were thawed and 0.2 ml. bovine serum albumin (2 mg./ml. in water) added as a co-precipitant, followed by 0.5 ml. ice-cold 1N-PCA. After mixing, the tubes were allowed to stand for 10 minutes at 0° before the addition of 2.5 ml. ice-cold distilled water. The precipitate was removed by centrifugation at 1,000g for 15 minutes. The extinction of the supernatant was read at 260 mµ in a Unicam SP 500 spectrophotometer.

4. The preparation of bentonite.

It has been reported that bentonite binds to and inhibits ribonucleases (Brownhill, Jones and Stacey, 1959) and also stabilises and protects tobacco mosaic virus RNA (Fraenkel-Conrat, Singer and Tsugita, 1961). Bentonite was therefore used in the extraction of RNA prior to sedimentation analysis (Section 2.5.). Suitable suspensions were prepared by suspending 2 g. bentonite

(The British Drug Houses Ltd., Poole, England) in 40 ml. water. The suspensions were centrifuged at 800g for 15 minutes. The sediment was discarded and the supernatant material was centrifuged at 8,700g for 20 minutes. The sediment so obtained was re-suspended in 0.1 M-EDTA, pH 7.0 and stored in this solution for 48 hours at room temperature. The material was then centrifuged once more at 800g. The sediment was again discarded and the supernatant centrifuged at 8,700g for 20 minutes. The sediment was suspended in 0.01 M-sodium acetate buffer, pH 6.0, centrifuged at 8,700g and the sediment taken up in the acetate buffer at a concentration of 2-6% (w/v).

5. The isolation of RNA prior to sedimentation analysis.

The method used was a modification of the phenol extraction technique of Kirby (1956). The reagents used were as follows:

A. 3% (w/v) bentonite in 0.01 M-sodium acetate buffer, pH 6.0.

B. 5% (w/v) aqueous sodium lauryl sulphate.

C. 0.01 M-sodium acetate buffer, pH 5.2.

D. Homogenising medium. A mixture of 0.7 ml. reagent A, 1.6 ml. reagent B and 5 ml. reagent C.

E. 90% (w/v) aqueous phenol containing 0.1% (w/v) 8-hydroxyquinoline. (8-hydroxyquinoline inhibits ribonucleases

and improves the yield of RNA (Kirby, 1962)).

F. 20% (w/v) aqueous sodium acetate.

G. Absolute ethanol.

H. "Buffer X." 0.01 M-tris-HCl buffer, pH 7.5, 0.001 M with respect to $MgCl_2$.

I. Bovine pancreatic deoxyribonuclease (Sigma Chemical Company, London, England).

J. "Buffer Y". 0.01 M-sodium acetate buffer, pH 5.2, 0.05 M with respect to NaCl and 0.001 M with respect to $MgCl_2$.

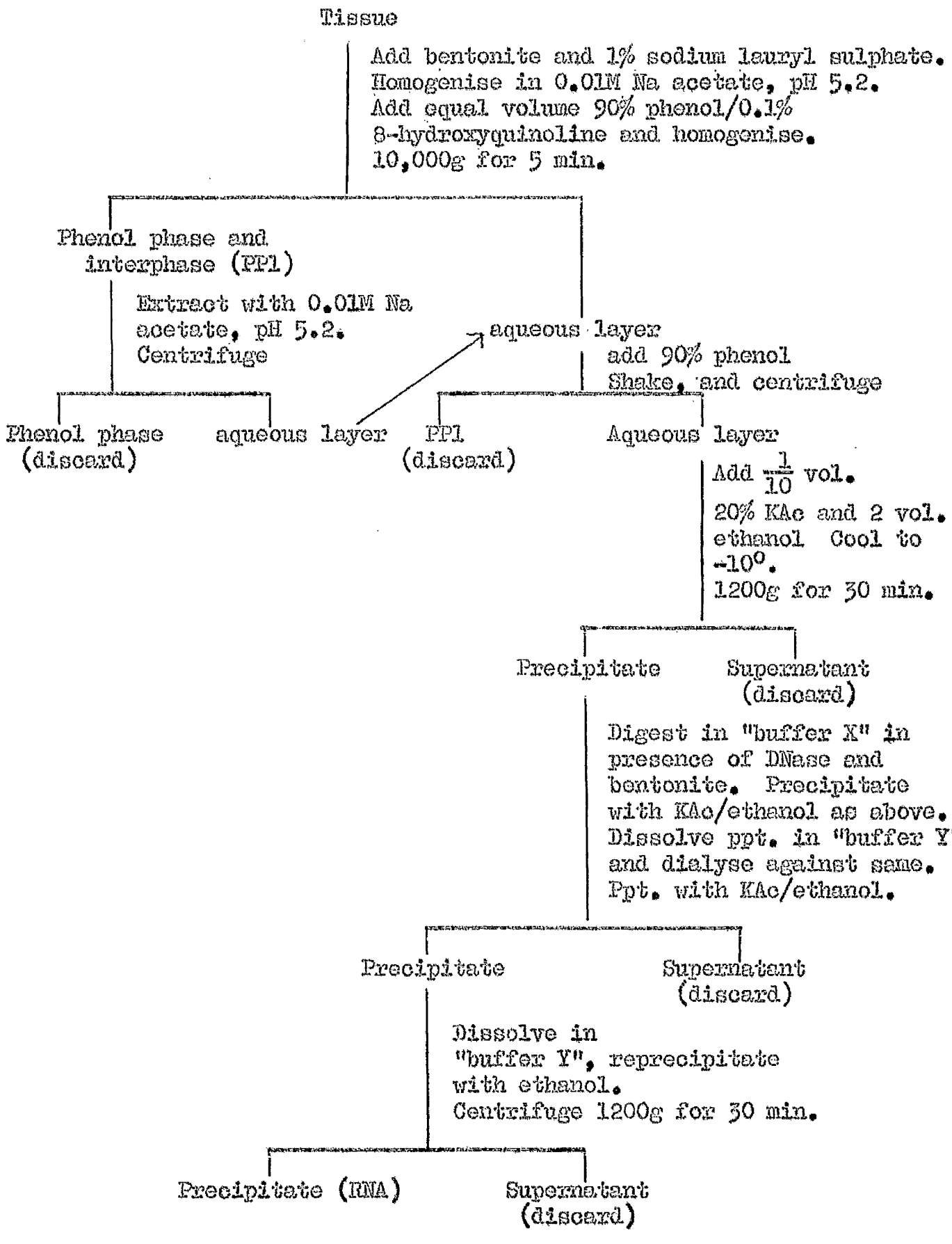
250-700 mg. tissue were suspended in 7.3 ml. homogenising medium (reagent D) and homogenised thoroughly in a Potter homogeniser with 5 passes of the pestle at full speed. The preparations were kept in ice throughout this and subsequent procedures. 8 ml. of 90% (w/v) aqueous phenol containing 0.1% (w/v) 8-hydroxyquinoline were added and the mixture homogenised again with 5 passes of the pestle at full speed.

The resulting emulsion was centrifuged at 10,000g for 5 minutes at 4° and the aqueous phase removed with a Pasteur pipette and mixed with a drop of 3% (w/v) bentonite in a chilled 50 ml. Quickfit flask. The phenol-interface residue was re-extracted with 4 ml. of 0.01 M-sodium acetate buffer, pH 5.2 by shaking for 10-15 minutes in a mechanical shaker at room temperature. After

centrifugation, the aqueous phase was removed and combined with the first. The combined aqueous extracts were shaken for 10 minutes with an equal volume of 90% phenol containing 0.1% 8-hydroxyquinoline and centrifuged at 10,000g for 10 minutes. The aqueous phase was removed to a 50 ml. graduated centrifuge tube and made 2% (w/v) with respect to sodium acetate. The RNA was precipitated by the addition of 2 volumes of ethanol precooled to -10° and left at -10° for 10 minutes. The RNA was collected by centrifugation at 1,200g for 30 minutes at -10° and dissolved in 4 ml. "buffer X" (reagent H). After addition of 60 µg. bovine pancreatic deoxyribonuclease, the solution was incubated for 10 minutes at 37°. The mixture was cooled on ice and shaken for 10 minutes with a half volume of 90% phenol containing 0.1% 8-hydroxyquinoline. The mixture was centrifuged at 10,000g for 10 minutes and the aqueous phase removed and made 2% (w/v) with respect to sodium acetate. The RNA was precipitated with 2 volumes of pre-cooled (-10°) ethanol and, after collection by centrifugation, was dissolved in 7 ml. "buffer Y" (reagent J). The solution of RNA was then dialysed against two changes of 7 litres of this buffer for 14-18 hours. The dialysed solution was centrifuged at 10,000g for 10 minutes to remove insoluble material and the RNA was precipitated with ethanol as before. The RNA precipitate was washed once more with 3 ml. of pre-cooled (-10°) absolute alcohol and after careful decanting and drying of the tube walls, dissolved in 2 ml. of "buffer Y". 2 ml. of

Figure 4

OUTLINE OF METHOD USED FOR ISOLATION OF RNA FOR SEDIMENTATION ANALYSIS



ethanol (-10°) was added and the mixture left at -10° for 30 minutes. The RNA was centrifuged down, the walls of the tube wiped and the RNA redissolved in 0.6 ml. "buffer Y". This was the final RNA preparation. A flow sheet summarising the extraction procedure is given in Figure 4.

6. Ultracentrifugal studies on RNA.

1. Sedimentation analyses of RNA.

Sedimentation analysis of RNA preparations were performed by centrifugation in linear sucrose density gradients. The gradients were prepared using the device shown in Figure 5. Screws C and D were closed, 2.25 ml. of 5% (w/v) sucrose in "buffer Y" pipetted into chamber A and 2.25 ml. of 25% (w/v) sucrose in "buffer Y" pipetted in chamber B. Screw C was opened, a gentle stream of air bubbled through the 25% sucrose in chamber B, screw D opened and the solution collected in a cellulose nitrate (2" x $\frac{1}{2}$") centrifuge tube. This procedure produced a linear gradient of sucrose concentration down the centrifuge tube.

0.1 to 0.3 ml. RNA in "buffer Y", containing about 0.5 mg. RNA was layered on top of the sucrose gradients and the tubes were centrifuged in the swinging bucket rotor (SW 39) of a Spinco Model L preparative ultracentrifuge for varying periods of time and at varying speeds as indicated in the legends to the appropriate figures. After centrifugation, the bottom of each tube was punctured with a 14 gauge hypodermic needle and the sucrose solution

FIGURE 5.

DEVICE USED TO PREPARE LINEAR SUCROSE CONCENTRATION GRADIENTS.

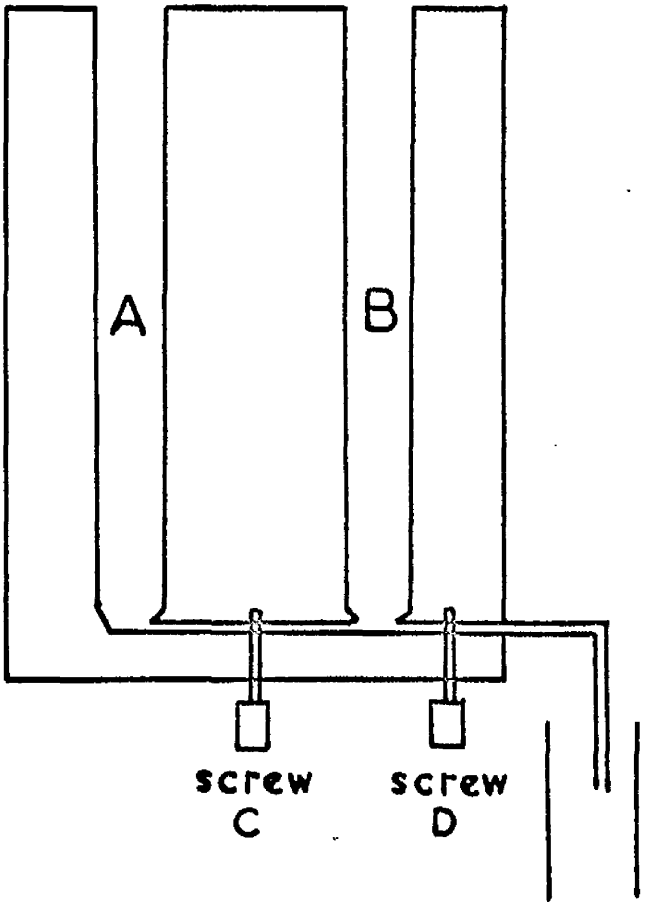
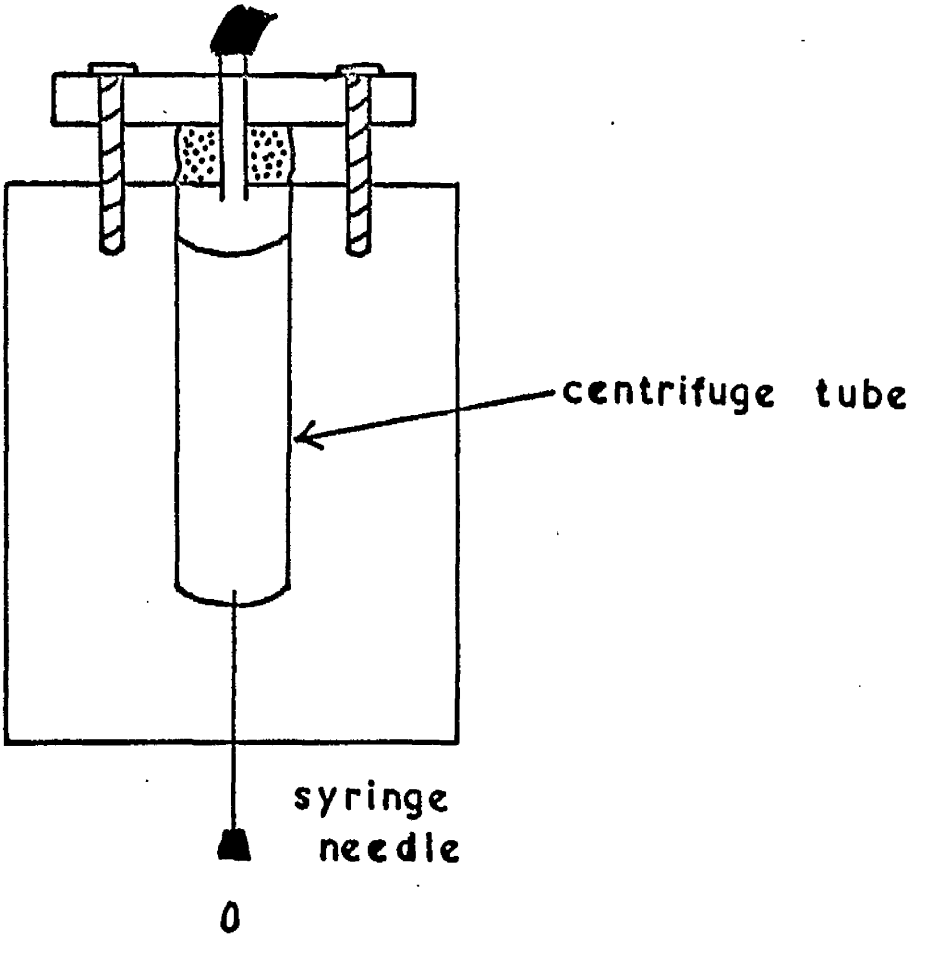


FIGURE 6.

DEVICE USED TO PUNCTURE THE BOTTOM OF A CENTRIFUGE TUBE PRIOR TO COLLECTION OF THE FRACTIONS.



collected in 2 drop fractions using the device shown in Figure 6. 0.6 ml. water was added to each fraction and the extinction at 260 mμ. read in 1 cm. microcells in a Unicam SP 500 spectrophotometer fitted with a micro-cell attachment. The fractions were then assayed for radioactivity (Section 2.7.).

6.2. Determination of sedimentation coefficients.

Solutions of RNA in 0.15 M-NaCl were centrifuged in the Spinco Model E analytical ultracentrifuge, equipped with an ultraviolet optical system, to determine the sedimentation coefficients of the various components. Runs were performed at 20° at 47,770 rev./min. Photographs were taken at 4 minute intervals. The sedimentation coefficient was calculated using the following equation:

$$W^2 S = \frac{d \log_e x}{dt}$$

where W = angular velocity (radians/second)

x = distance of boundary from centre of rotation (cm.).

t = time (sec.).

S = sedimentation coefficient.

7. Assay of radioactivity.

Tritium labelled samples were assayed in a three-channel Nuclear Chicago model 725 liquid scintillation spectrometer or a two-channel Packard Tricarb liquid scintillation spectrometer. The scintillator used consisted of 0.7% (w/v) 2,5-diphenyloxazole, 0.03% (w/v) 1,4-bis-[2-(5-phenyloxazolyl)]-benzene, 10% (w/v)

naphthalene (Nuclear Enterprises, Edinburgh, Scotland) dissolved in Analar grade dioxane. It was freed from peroxides by passage through a column of activated alumina (Type "A", 16/32 mesh, Peter Spence & Sons Ltd., Widnes, England) under nitrogen. For assay, 8 ml. of scintillator was added to 0.8 ml. of an aqueous solution of the sample.

8. Analysis of blood.

Blood was obtained from the tail vein of rats under ether anaesthesia.

1. Haematocrit.

Blood haematocrits were obtained using the Hawksley micro-haematocrit centrifuge (Hawksley and Sons Ltd., Lancing, England). Blood was drawn directly into capillary tubes by capillarity. The unfilled end of the tube was sealed in a bunsen flame and the tubes were centrifuged for 5 minutes. The percentage packed cell volume was read on a Hawksley micro-haematocrit reader, giving an indication of the state of dehydration of the animal.

8.2. Estimation of serum sodium and potassium.

The reagents used were as follows:

A. Stock sodium solution: 58.5 g. NaCl/l.

B. Stock potassium solution: 7.46 g. KCl/l.

C. Standard sodium solution: 75 ml. stock sodium solution and 25 ml. stock potassium solution made up to 500 ml.

with distilled water. For use, this solution was diluted 1 in 1000 with distilled water to give a concentration of 150 milli equivalents sodium ion per litre.

D. Standard potassium solution: 70 ml. stock sodium solution and 20 ml. stock potassium solution made up to 500 ml. with distilled water. For use this solution was diluted 1 in 50 with distilled water to give a concentration of 4 milli-equivalents potassium ion per litre.

The serum, diluted 1 in 1000 for sodium estimation and 1 in 50 for potassium estimation, was sprayed into the flame of an EEL flame photometer (Evans Electroselenium Ltd., Halstead, England). Using the appropriate filter, the light output was compared with the light output from the corresponding standard solution.

8.3. Estimation of serum chloride.

Serum chloride was estimated by the method of Schales & Schales (1941). The reagents used were as follows:

A. 1N-nitric acid.

B. Diphenylcarbazone indicator. 100 mg. diphenylcarbazone was dissolved in

100 ml. 95% (v/v) alcohol and stored in the dark in the refrigerator.

C. Mercuric nitrate. 2.95 g. mercuric nitrate was dissolved in a few hundred ml. distilled water. 20 ml. 2N-nitric acid was added, and the volume made up to 1 l. with distilled water.

D. Standard chloride solution. 585 mg. sodium chloride were dried at 120° and dissolved in 1 l. distilled water.

1.8 ml. distilled water, 0.6 ml. diphenylcarbazone indicator and 0.02 ml. 1N-nitric acid were added to 0.2 ml. serum in a test tube. The mixture was titrated with standard mercuric nitrate using a 2 ml. microburette, the end-point being the sudden appearance of a persistent faint violet colour. The titration was repeated using 2 ml. of the standard chloride solution.

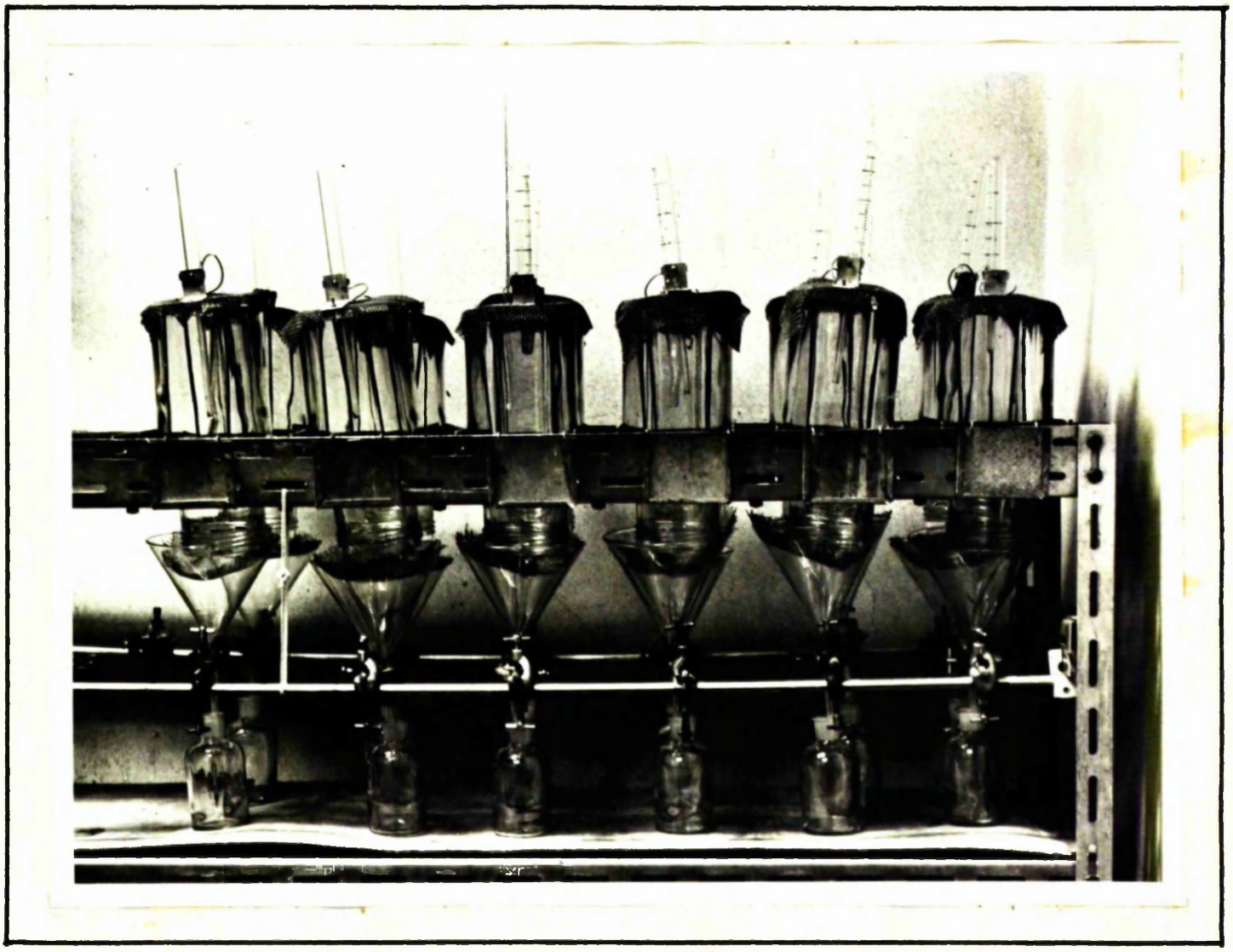
9. Collection and analysis of urine.

1. Collection of urine.

During the experimental periods the animals were housed in metabolism cages (Thomson and Munro, 1955) in an air-conditioned room at constant temperature and humidity. The cages consisted of oval glass jars with the bottoms removed, held in the inverted position in a structure built of "Dexion". A removable wire mesh arrangement provided floor and top of the cage and allowed the

FIGURE 7.

METABOLISM CAGES.



animals to be kept in comfort and to be easily accessible. A filter funnel placed below the neck of the glass jar, with a lid of wire gauze, allowed urine to pass through but retained faeces and cast hairs (Figure 7). The urine was collected in 24 hour periods in 250 ml. reagent bottles placed below the filter funnels. As a preservative, 10 ml. of 6N-HCl was added to the bottles except when urinary ammonia was to be estimated, when 1 ml. chloroform was used instead.

9.2. Estimation of urinary urea.

Urinary urea was estimated by the hypobromite method using the Doremus Ureometer (Figure 8). The tube A was filled with sodium hypobromite solution (a mixture of 10 ml. bromine and 100 ml. 40% (w/v) sodium hydroxide) by pouring this into the bulb B and tilting the instrument so as to release air bubbled from A. 1 ml. of urine was then cautiously admitted to A from the graduated sidearm C. After 15-20 minutes, the concentration of urea was read off tube A which is graduated in grams of urea.

9.3. The estimation of urinary ammonia.

The reagents used were as follows:

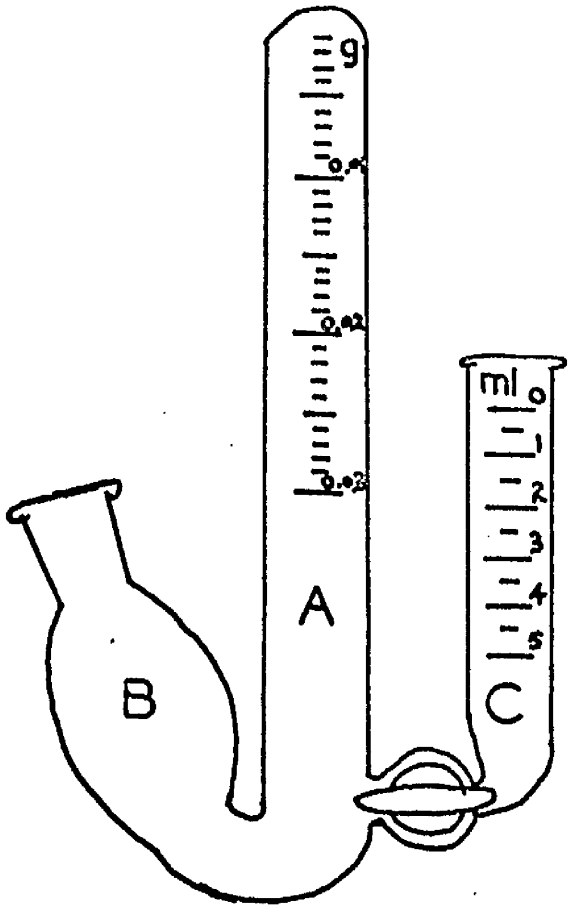
A. Phenolphthalein indicator.

B. 0.1N-NaOH.

C. Neutralised formaldehyde solution.
1 ml. of 40% (w/v) formaldehyde was neutralised with 0.1N-NaOH.

FIGURE 8.

THE DOREMUS UREOMETER.



2 ml. of urine was diluted 1 in 10 with distilled water. Two drops of phenolphthalein were added and 0.1N-NaOH from a burette until a stable pink colour was obtained. Neutralised formaldehyde solution was added and the mixture titrated with 0.1N-NaOH to the same pink colour as before. Since in this formol titration one NH_2 group yields one equivalent of hydrogen ion, then 1 ml. 0.1N-NaOH = 1.4 mg. ammonia.

10. Statistical analysis.

The statistical significance of the difference between means was assessed either by Student's "t" test or by analysis of variance. (Snedecor, 1946). Correlation coefficients between two groups of results and the significance of the correlation coefficients were obtained as described by Fisher (1954). The expressions $P < 0.05$ and $P < 0.01$ etc. are used in the conventional sense to indicate significance at the 5% and 1% levels etc. respectively. (Snedecor, 1946).

Section 3

R E S U L T S

R E S U L T S.

1. The normal rat kidney.

Although the problem of compensatory renal hypertrophy has been studied for over a hundred years, as the review of the literature in the Introduction shows, the general picture is still very confused. There is little agreement about the mechanism controlling the process or even about the magnitude of the changes involved. Before investigating the process or the mechanism controlling it, it was clearly necessary to obtain a suitable means of measuring the growth of the kidney. The methods used by previous workers were not very satisfactory. They involved comparison of the size, mitotic activity or composition of the kidney removed at death with that of the kidney removed at operation. Since these measurements were not, in general, very precise or reliable, it seemed essential to begin by reviewing all techniques. The first step in the present investigation therefore, was necessarily a careful and detailed comparison of the right and left kidneys of normal animals. The confusion prevailing in the literature on this subject is well illustrated by the uncertainty, particularly in the rat, about the fundamental question as to whether the two kidneys of normal animals are equal in weight. Of the early workers, Arataki (1926a) found that between birth and 350 days of age, the right kidneys of male rats were, on average, 2.1% heavier and of female rats 2.3% heavier than the left kidneys. Smith and Moise

(1927), however, reported that any difference there may have been in the weight of the two kidneys of male rats was so slight that it could be disregarded. More recently, Zumoff and Pachter (1964) and Mason and Ewald (1965) found no significant difference between the weights of the two kidneys in rats. In female mice, on the other hand, Rosen and Cole (1960) and Berech and Curtis (1964) have reported that the right kidney was, on average, about 5% heavier than the left. In dogs, Allen, Bollman and Mann (1935) also found that the right kidney was about 5% heavier than the left and Astarabadi and Essex (1953) reported that in most healthy dogs, the right kidney was always the heavier. In rabbits and in man, however, Arataki (1926) reported that the right kidney was smaller than the left. To clarify this point, and as a basis for future experiments, a fresh study was made of the size and composition of the kidneys of normal rats.

1.1. Kidney weight.

The question whether one kidney is normally heavier than the other is of particular importance because the earliest and the most used method of following the growth of the remaining kidney has been to compare its weight at death with that of the kidney removed at operation. This immediately raises the question of how far the weight of the excised kidney is affected by the way in which it was removed from the animal. It is not possible to remove both kidneys under exactly the same conditions; as soon as one kidney is excised,

the conditions are altered and it is possible that the weight of the surviving kidney may then be affected, say, by a redistribution of blood in the operated animal. Table 10 shows the weights of the kidneys following removal by three different procedures. In the first, the animals were anaesthetised with ether and the right kidneys ligated as they would have been in the normal operation of unilateral nephrectomy, and then excised. The animals were then killed, as they might have been at the end of an experiment, by cutting the inferior vena cava and aorta and allowing them to bleed to death, still under ether anaesthesia. The left kidneys were then excised. Table 10 shows that the right kidneys, which were removed first, were significantly heavier than the left. This may reflect a real difference which exists in vivo. It is possible, however, that it was due to the kidney excised from the living animal containing more blood than its partner which was removed after exsanguination. To check this point, a second group of rats were killed by exsanguination under ether anaesthesia. Both kidneys were then removed from the dead animals and weighed. Table 10 shows that in this group also, the right kidney was significantly heavier than the left. The difference in weight is therefore real, and not an artefact. Moreover the difference between right and left kidneys was not significantly greater in one group than in the other. It is not likely therefore, that the way in which the kidneys are excised has much effect on their weights. In the study of the effect of

Table 10

The effect on kidney weight of the method of removal.

Rat body weight (g.)	Method of removal of kidneys.	Right kidney weight (mg.)	Left kidney weight (mg.)	Difference in weight between right and left kidneys. (mg.)
262 ± 3.9	Right kidney ligated and excised; rat killed by exsanguination and then left kidney excised.	869 ± 32.3	785 ± 28.5	84 ± 20.3*
262 ± 8.0	Rat exsanguinated and both kidneys then excised.	852 ± 36.2	792 ± 31.7	60 ± 7.7*
262 ± 5.3	Right kidney ligated and excised; then left kidney ligated and excised in the same way.	850 ± 23.7	795 ± 26.4	55 ± 9.2*

Values are means for 10 animals ± S.E.M.

* Significantly different from zero with a P value of 0.001 or less.

The differences shown in the column on the extreme right are not significantly different from one another ($P > 0.05$).

unilateral nephrectomy, however, it is obviously not possible to exsanguinate the animal before removing the first kidney. At operation, one kidney will have to be ligated before excision. The animal will then be left for some period of time before removal of the second kidney. Accordingly, in a third group of animals, the right kidney was ligated and excised to simulate a unilateral nephrectomy. The left kidney was then immediately ligated and excised in exactly the same way. As Table 10 shows, the right kidney was again significantly heavier than the left. In addition, the difference in weight between the two kidneys was not significantly different from that found with the two previous methods. Since, however, this third method involves removal of the two kidneys in the manner required for unilateral nephrectomy, it was used as a standard procedure in all subsequent studies.

Although, from the results shown in Table 10, it seemed unlikely that the method of removal of the kidneys affected their weight, it seemed desirable to exclude any possible chance of error from this source. Accordingly, the effect of the order of removal of the kidneys on the observed kidney weight was investigated. Twenty rats were divided randomly into two groups. The kidneys were removed from the animals by the standard procedure quoted above. From one group, the right kidney was removed first and from the other group, the left kidney was removed first. The results are shown in Table 11. Irrespective of the order of removal of the kidneys, the right

Table 11.

The effect on kidney weight of the order of removal of the kidneys.

Rat Body Weight (g.)	Order of Removal of Kidneys	Right Kidney Weight (mg.)	Left Kidney Weight (mg.)	Difference in Weight between Right and Left Kidneys. (mg.)
272 ± 4.4	Right First	831 ± 22.5	772 ± 21.8	59 ± 11.8*
272 ± 4.1	Left First	858 ± 19.9	810 ± 23.8	47 ± 8.7*

Results are given as mean values ± S.E.M. for 10 animals.

* Significantly different from zero with a P value of 0.001 or less.

The differences shown in the column on the extreme right are not significantly different from one another ($P > 0.05$).

kidney was significantly heavier than the left. Moreover, the difference in weight was not significantly different in the two groups of animals. It can therefore safely be concluded that the right kidney of male rats of the strain and body weight used, is, on average, about 60 mg. (8%) heavier than the left. The results in Table 12 show that in female rats the weights of the right and left kidneys are almost exactly the same as in male rats of the same body weight. This is in contrast to the liver where a sex difference exists. The liver of male rats is about 10 to 20% heavier than that of females (Thomson, Heagy, Hutchison and Davidson, 1953). The difference in weight between right and left kidneys is not restricted to the rat weight range so far studied. Figure 9 shows that in the weight range 120 to 330 g. the right kidney was heavier than the left in 111 of 121 cases. Thus the two kidneys of the normal rat are not the same and the difference in weight will have to be taken into account when comparing the weight of the surviving kidney with that of the kidney removed at unilateral nephrectomy.

It was clear from the distribution of points in Figure 9 that the weight of one kidney bore a relationship to the weight of the other. A straight line was drawn through the points by the method of least squares and the correlation coefficient calculated. The very high value found (0.97) represents a good correlation. Clearly, therefore, although the two kidneys are not equal in weight, if the weight of one is known, the weight of the other can be calculated

Table 12

The effect of sex on kidney weight.

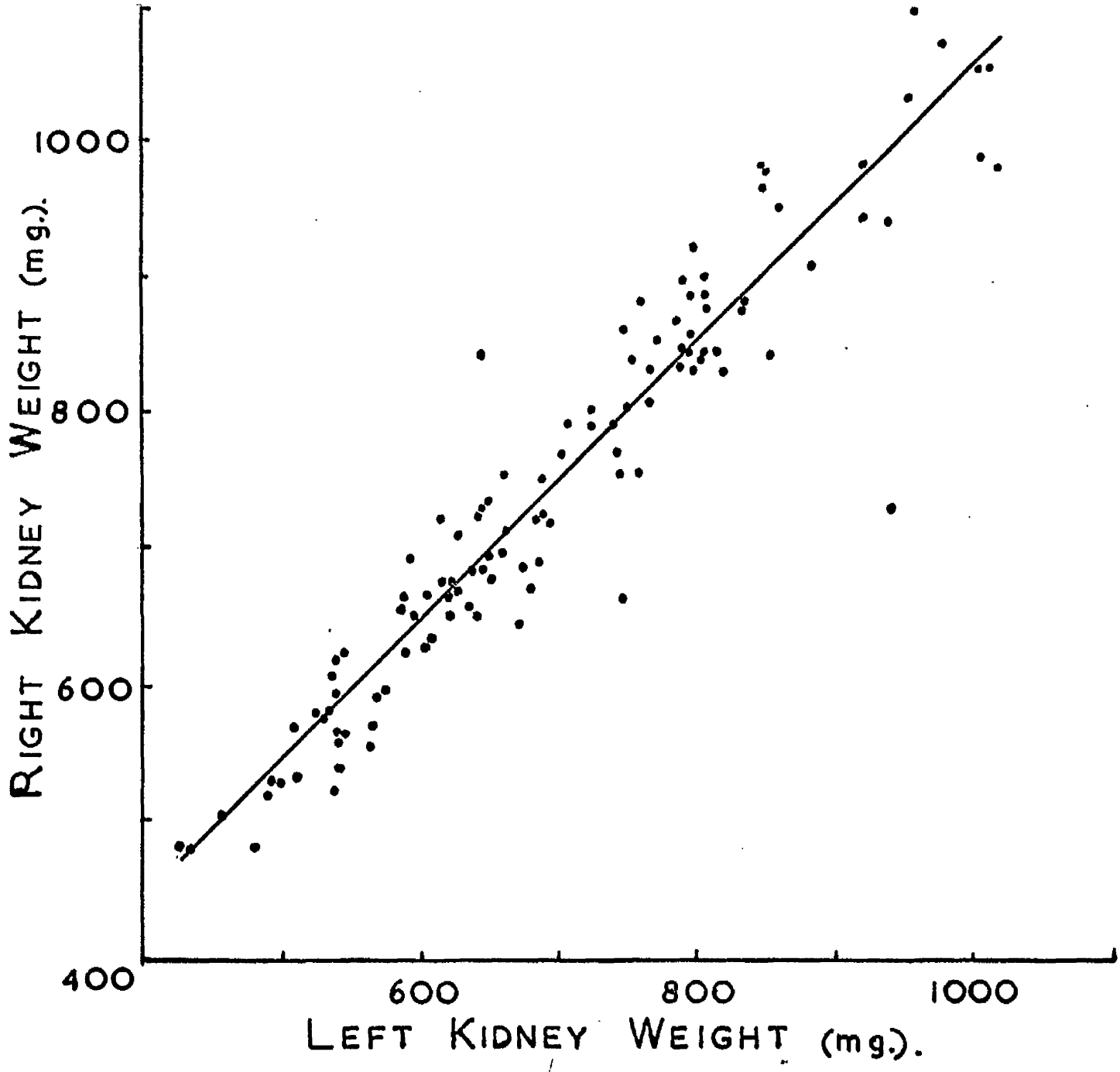
Sex	Rat Body Weight (g.)	Right Kidney Weight (mg.)	Left Kidney Weight (mg.)	Difference in Weight between Right and Left Kidneys.
Male	166 ± 3.9	627 ± 15.3	577 ± 15.4	50 ± 4.5*
Female	166 ± 3.8	620 ± 19.2	566 ± 19.9	54 ± 9.9*

Values are means ± S.E.M. for 10 animals.

* Significantly different from zero with a P value of 0.001 or less.

The differences in the kidney weight in the two groups are not significantly different ($P > 0.05$).

FIGURE 9.


1000
800
600
400
RIGHT KIDNEY WEIGHT (mg.).
600
800
1000
LEFT KIDNEY WEIGHT (mg.).

with a fair degree of confidence. Some workers have expressed their results in terms of the ratio of kidney weight to the body weight at the time of removal of the organ. Such a ratio can only have meaning if the weight of the kidneys in normal animals bears a fairly close relationship to body weight. Figure 10 shows that there was indeed a fair degree of correlation between right kidney weight and body weight in the weight range studied. Braun-Menéndez (1952), Benitz, Moraski and Cummings (1961) and Zumoff and Pachter (1964) have obtained similar results. The correlation coefficient obtained in the present observation (0.91) was however substantially lower than that obtained from Figure 9. In other words, the relationship between left kidney weight and right kidney weight was closer than that between right kidney weight and body weight. Figure 11 shows the relationship between total kidney weight and body weight. The correlation coefficient of 0.94 was slightly better than that obtained for right kidney weight against body weight.

It is noticeable that as body weight increases from 150 to 300 g. (an increase of 100%) the average weight of the right kidney increases from 590 mg. to 957 mg. (an increase of only 60%). In other words, large adult rats have smaller kidneys in proportion to their size than young adults. On the other hand, by using the formula

$$\text{surface area} = 12.54 \times \text{body weight}^{0.60} \text{ sq. cm.}$$

(Lee, 1929), it can be calculated that a 300 g. rat has only 52% more surface area than a 150 g. rat. It can therefore be argued

Figure 10

The correlation between right kidney weight and body weight of normal male rats.

The equation of the line is $y = 2.45x + 222$ and $r = 0.91$ ($P < 0.001$).

FIGURE 10.

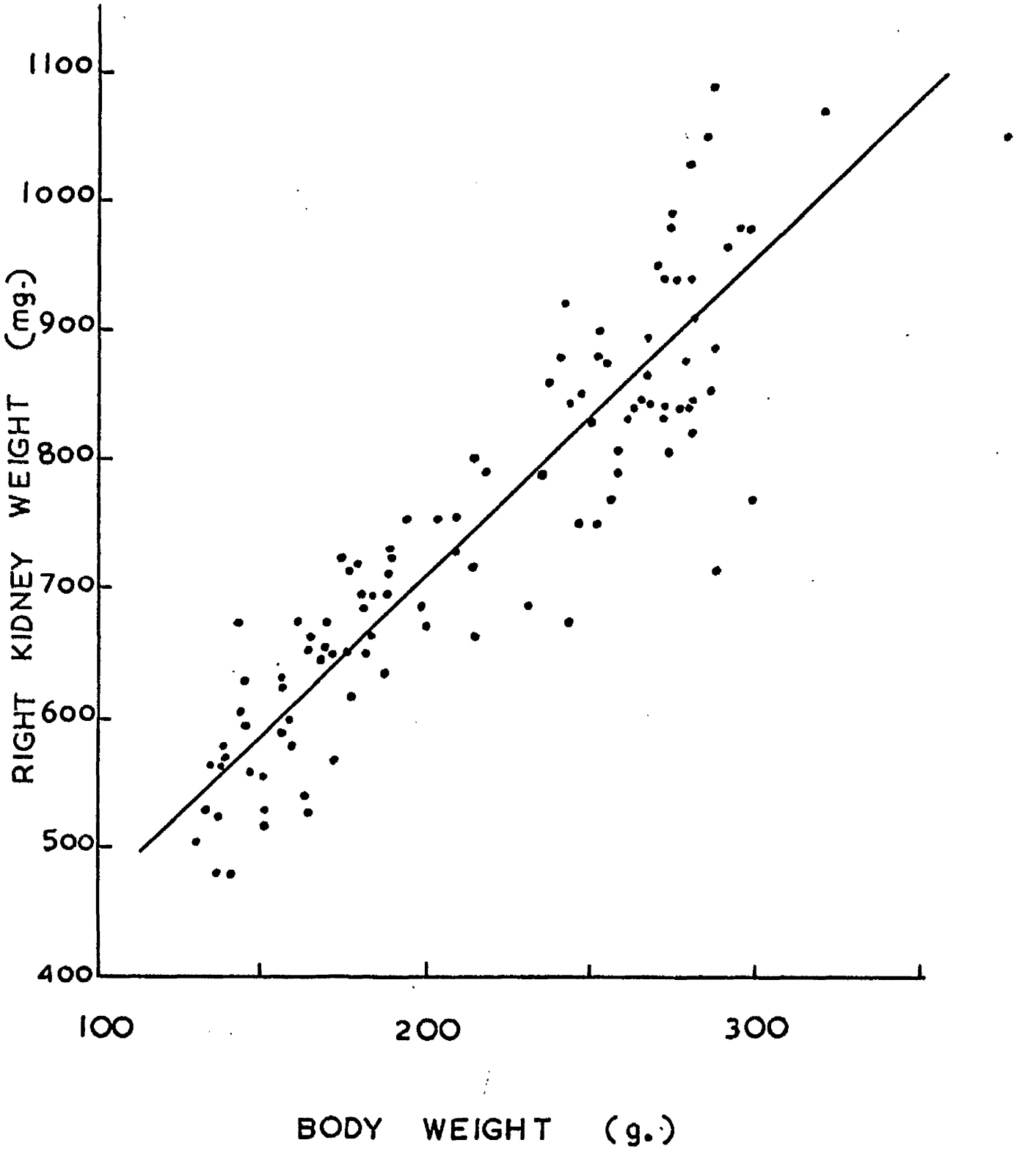
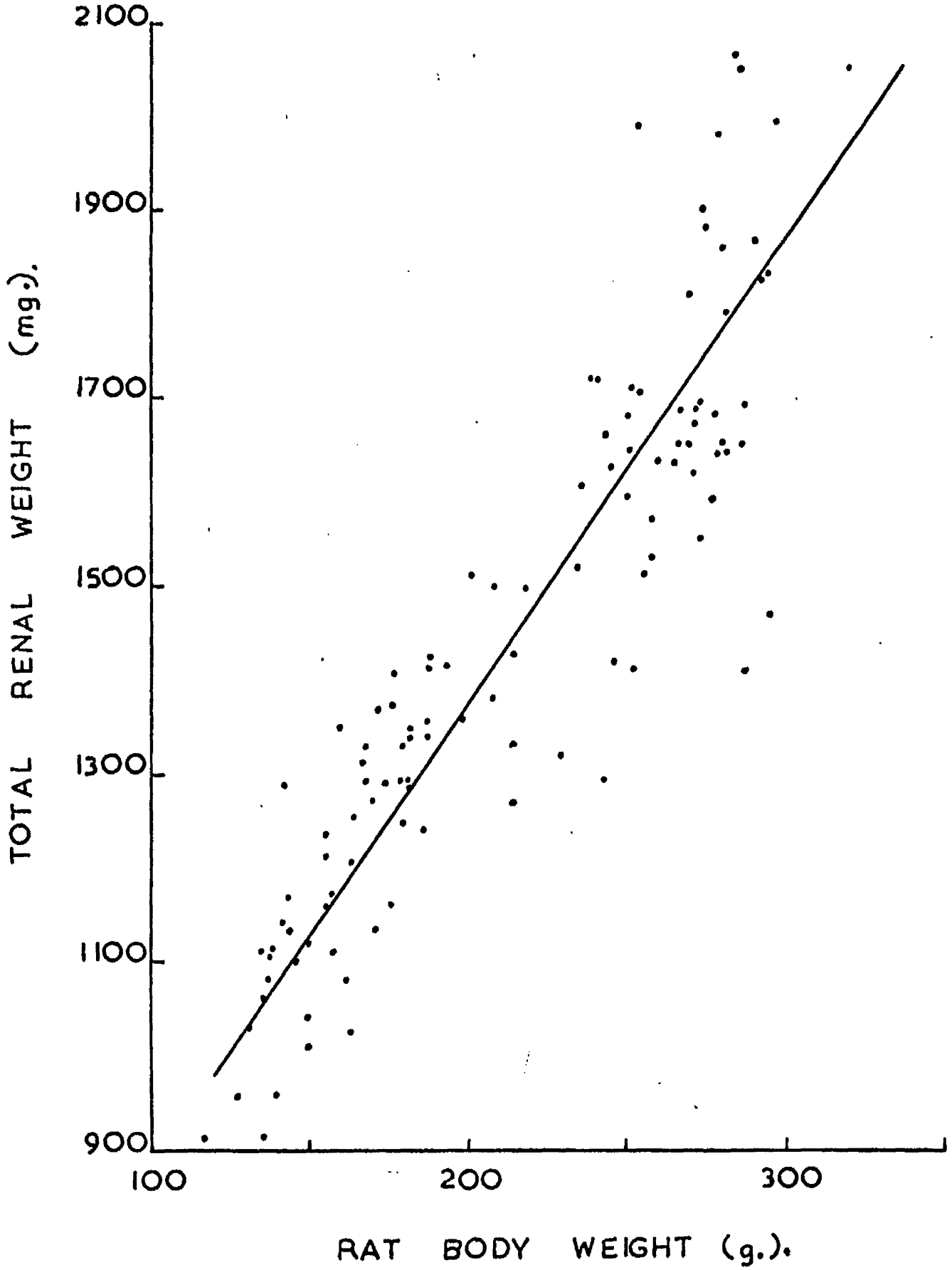

1100
1000
900
800
700
600
500
400
RIGHT KIDNEY WEIGHT (mg.)
100
200
300
BODY WEIGHT (g.)

Figure 11

The correlation between total renal weight and body weight of normal male rats.

The equation of the line is $y = 4.92x + 390$ and $r = 0.94$ ($P < 0.001$).

FIGURE 11.


2100
1900
1700
1500
1300
1100
900
TOTAL RENAL WEIGHT (mg.).
100
200
300
RAT BODY WEIGHT (g.).

that kidney size is more nearly proportional to surface area than to body weight. This is perhaps what one might expect if kidney size were determined ultimately by the nutritional requirements of the individual, since these are known to be related more directly to surface area than to body weight.

Since the body weight of animals can vary according to the nutritional state or to environmental factors, it seemed possible that better correlation might be found between renal weight and the weight of another organ such as the liver than between renal weight and body weight. A study was therefore made of the relationship between total renal weight and liver weight. The results are shown in Figure 12. The correlation was in fact, if anything, lower than between renal weight and body weight.

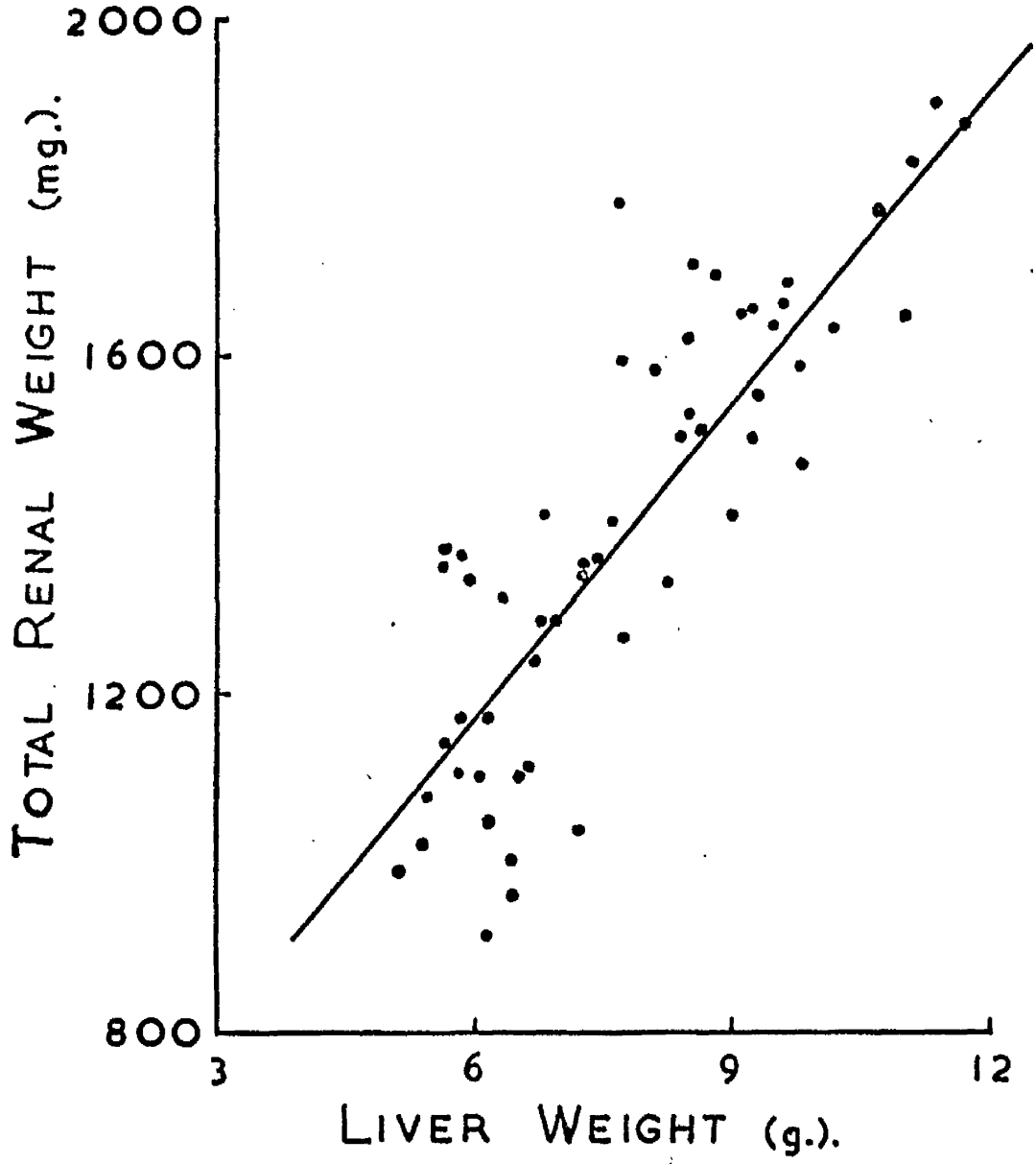
To sum up these observations. Though the two kidneys in the rat differ on the average quite substantially in weight, the relationship between their weights is fairly constant and certainly closer than the relationship of kidney weight to either body weight or liver weight. In practical terms this means that the growth of the surviving kidney after unilateral nephrectomy can be conveniently and fairly reliably measured by comparing its weight with that of its partner removed at operation. Such a comparison will generally be more reliable than an attempt to relate the weight of the surviving kidney to body weight (or to the weight of, say, the liver). It must however take account of the difference in weight between the two

Figure 12

The correlation between total renal weight and liver weight of normal male rats.

The equation of the line is $y = 125x + 426$ and $r = 0.85$ ($P < 0.001$).

FIGURE 12.


2000
1600
1200
800
TOTAL RENAL WEIGHT (mg.).
3
6
9
12
LIVER WEIGHT (g.).

kidneys before operation. Accordingly, in the present experiments, a standard procedure was adopted in which the right kidney was ligated and excised in all unilateral nephrectomies (unless otherwise stated). At the end of each experiment, the left kidney was always ligated and excised under ether anaesthesia in exactly the same way. The two kidneys, therefore, were, as far as possible, treated exactly alike.

1.2. Kidney composition.

In order to define more clearly the differences between right and left kidneys brought out by the differences in weight, a comparison was made of their protein and nucleic acid contents. These particular tissue components were chosen because of their special relationship to growth. Thus an increase in the protein content of the kidney might be expected to be a more sensitive and reliable indication that kidney growth had taken place than an increase in kidney weight, since the latter is liable to error from variation in content of water and fat. The RNA content might prove to be an indicator of growth in a different sense, since there is ample reason to think that it would increase during and immediately before the actual process of growth. Finally the content of DNA would, since the amount of DNA per nucleus in the kidney is constant, give a measure of cell number. Moreover, by relating other tissue components to DNA, it should be possible to obtain an estimate of mean cell mass and composition. For example, the DNA concentration per 100 mg. tissue is inversely proportional to the mass of tissue

per cell and the ratios of protein/DNA and RNA/DNA are directly proportional to the amounts of protein and RNA per cell. This sort of rough calculation takes no account, of course, of extracellular material, and this must be borne in mind in assessing its significance.

Table 13 shows the results obtained in a comparison of the two kidneys of normal rats. The inequality in organ weight was the most marked difference, the right kidney being about 10% heavier than the left. It contained about 7% more RNA ($P < 0.01$) and DNA ($P < 0.02$). Although it also contained about 5% more protein than the left kidney, this difference was not significant ($P > 0.05$). On the assumption, outlined above, that the DNA content per cell is constant, this means that the right kidney contains about 7% more cells than the left. The average protein and RNA contents per cell were, however, the same for both kidneys. Indeed the most striking generalization to emerge from this experiment was that while the average RNA/DNA ratio varied a good deal from animal to animal, there was very little difference, if any, in this ratio between the right and left kidneys of a single animal. The difference was never more than 2%. In practical terms, this means that any changes in RNA/DNA ratio in the surviving kidney will be detected much more readily by comparing it with its partner excised at operation than with the kidneys of a control group of unoperated animals. There was a greater scatter in the protein per cell and in mean cell mass (estimated from the reciprocal of DNA/100 mg.) between the right and left kidneys, perhaps because these would

Table 13

The weight and composition of right and left kidneys of male rats.

	Right Kidney	Left Kidney	Ratio $\frac{\text{Left}}{\text{Right}}$
Weight (mg.)	955 ± 29.5	861 ± 28.0	0.90 ± 0.011*
DNAP			
μg/100 mg. kidney	29.1 ± 0.80	30.2 ± 0.64	1.04 ± 0.017
μg/kidney	277 ± 8.1	259 ± 5.8	0.94 ± 0.018‡
RNAP			
μg/kidney	413 ± 12.6	385 ± 10.2	0.93 ± 0.017†
μg/μg DNAP	1.49 ± 0.02	1.49 ± 0.02	1.00 ± 0.004
Protein			
mg/kidney	138 ± 3.4	131 ± 1.2	0.95 ± 0.044
μg/μg DNAP	521 ± 13.9	517 ± 9.9	0.99 ± 0.035

Values are means ± S.E.M. for 9 animals weighing between 260 and 280 g.

* Ratio significantly different from unity with a P value of 0.001 or less.

† Ratio significantly different from unity with a P value of 0.01 or less.

‡ Ratio significantly different from unity with a P value of 0.02 or less.

be affected by the blood content of the kidneys.

The values obtained for the RNA and DNA contents of the kidney by other workers have varied considerably. Mandel, Mandel and Jacob (1951) and Thomson et al. (1953) have obtained values for the RNA/DNA ratio in adult rat kidney of over 2.0, whereas Schneider (1946) and Rose and Schweigert (1952) obtained values of less than 1.0. More recently, Kurnick (1955), Munro (1964) and Lotspeich (1965) have obtained values for the same ratio of between 1.4 and 1.8 which are more in agreement with the results of Table 13. The differences in the RNA/DNA ratio must be due to the determination of RNA, since there is less variation in the values obtained for DNA concentrations. These may have arisen as a result of different methods of extracting and estimating the nucleic acids. When the nucleic acids have been extracted by the procedure of Schmidt and Thannhauser (1945), the results obtained have always been higher than after extraction by the Schneider method (Schneider, 1945). Although a similar variation has been found with estimations on other tissues, it is clear that the RNA/DNA ratio is much higher for liver and pancreas than for kidney, whereas the ratio for tissues such as small intestine, lung, thymus and bone marrow has, in general, been found to be lower than in kidney (Leslie, 1955). It seems likely that the value for the RNA/DNA ratio is related to the protein synthesising capacity of the tissue, particularly to its activity in synthesising protein for export. Tissues synthesising

large amounts of protein, such as liver and pancreas, have a high RNA/DNA ratio whereas tissues which do not require to synthesise much protein such as lung, or which are mainly concerned with proliferation, such as bone marrow, have a low RNA/DNA ratio. It seems reasonable that kidney should occupy an intermediate position.

The difference in size and composition between the two kidneys of the same animal seemed therefore to have been clarified by making use of the fact that all kidney cells have the same DNA content. It seemed worthwhile to see whether the same sort of approach would throw more light on the relationship between kidney size and body weight. Figure 13 shows the relationship between the total content of DNA of the right kidney and the body weight of the animal. In increasing in body weight from 150 to 300 g., there was an increase of about 90% in the DNA content of the kidney. The correlation coefficient obtained ($r = 0.81$) perhaps surprisingly, indicates that the relationship between kidney cell number and body weight is not as close as that between kidney weight and body weight (Figure 10). The increase in total content of RNA (Figure 14) and protein (Figure 15), in the same body weight range, were about 100% and 75% respectively. The correlation between each of these constituents and body weight was of the same order as between DNA content and body weight.

Figures 16, 17 and 18 show the relationship between DNA concentration, RNA per cell and protein per cell on the one hand and

Figure 13

The correlation between total DNAP content of the right kidney and body weight of normal male rats.

The equation of the line is $y = 0.95x + 22$ and $r = 0.81$ ($P < 0.001$).

FIGURE 13.

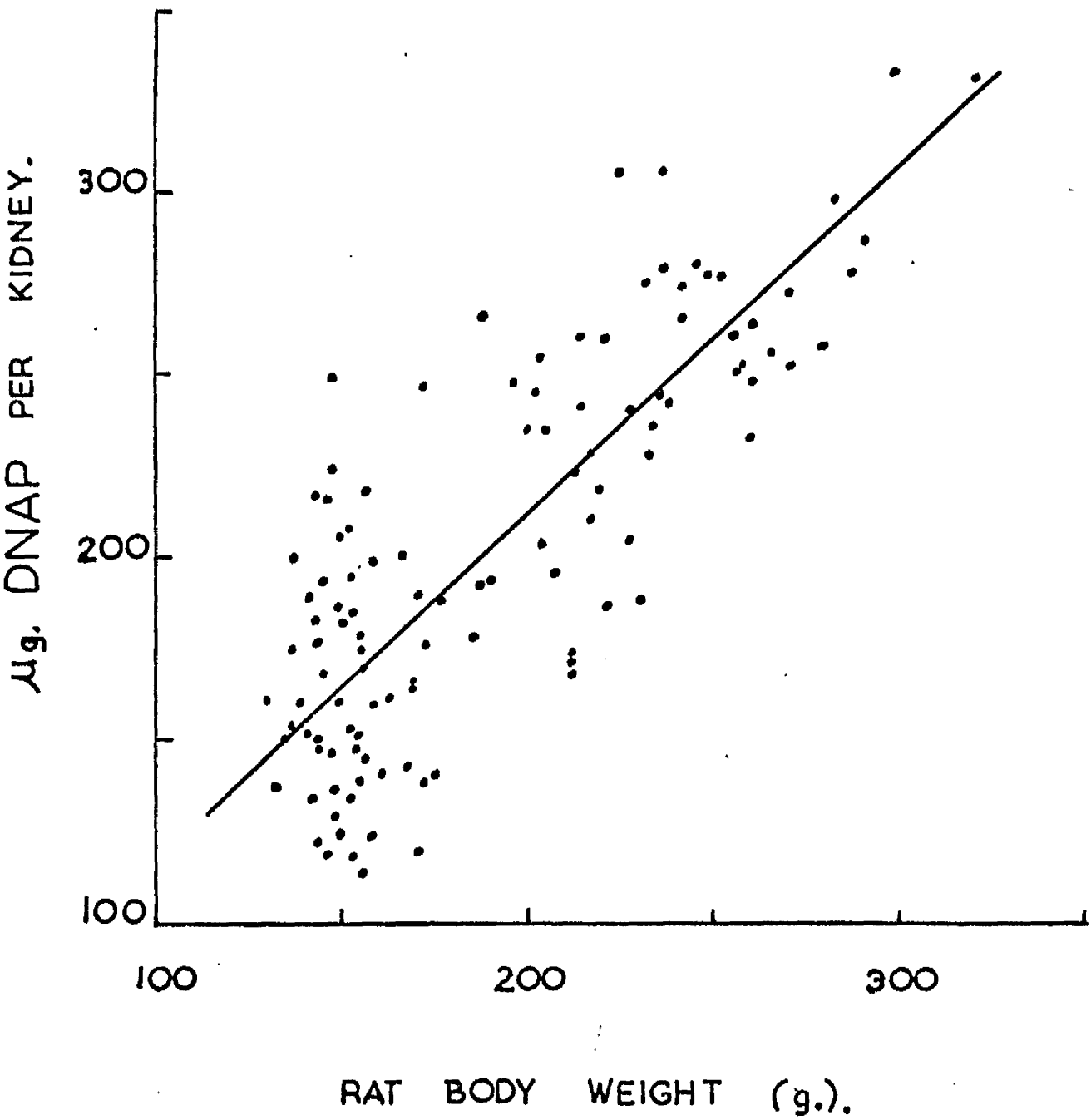


Figure 14

The correlation between total RNAP content of the right kidney and body weight of normal male rats.

The equation of the line is $y = 1.41x + 27$ and $r = 0.81$ ($P < 0.001$).

FIGURE 14.

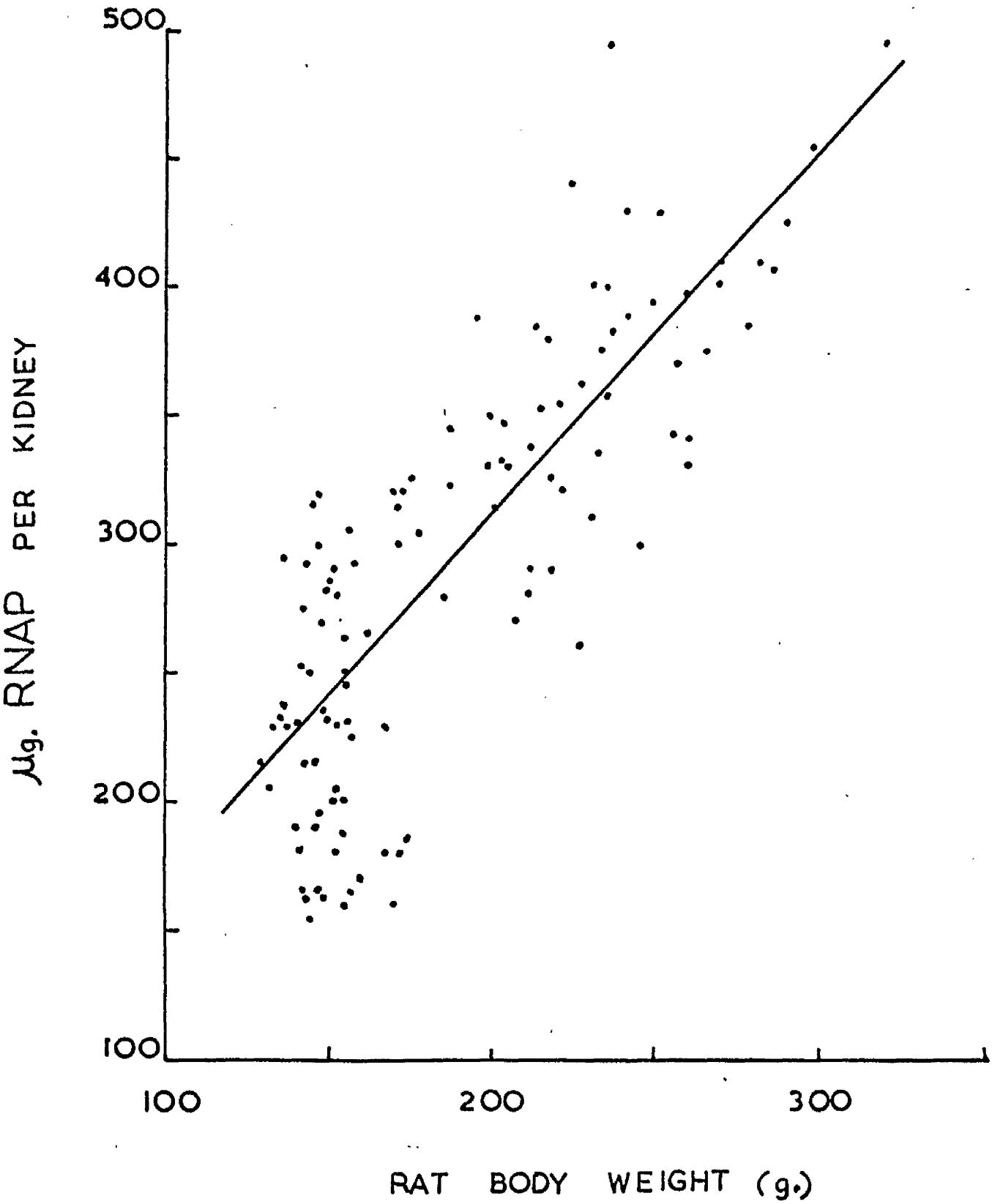
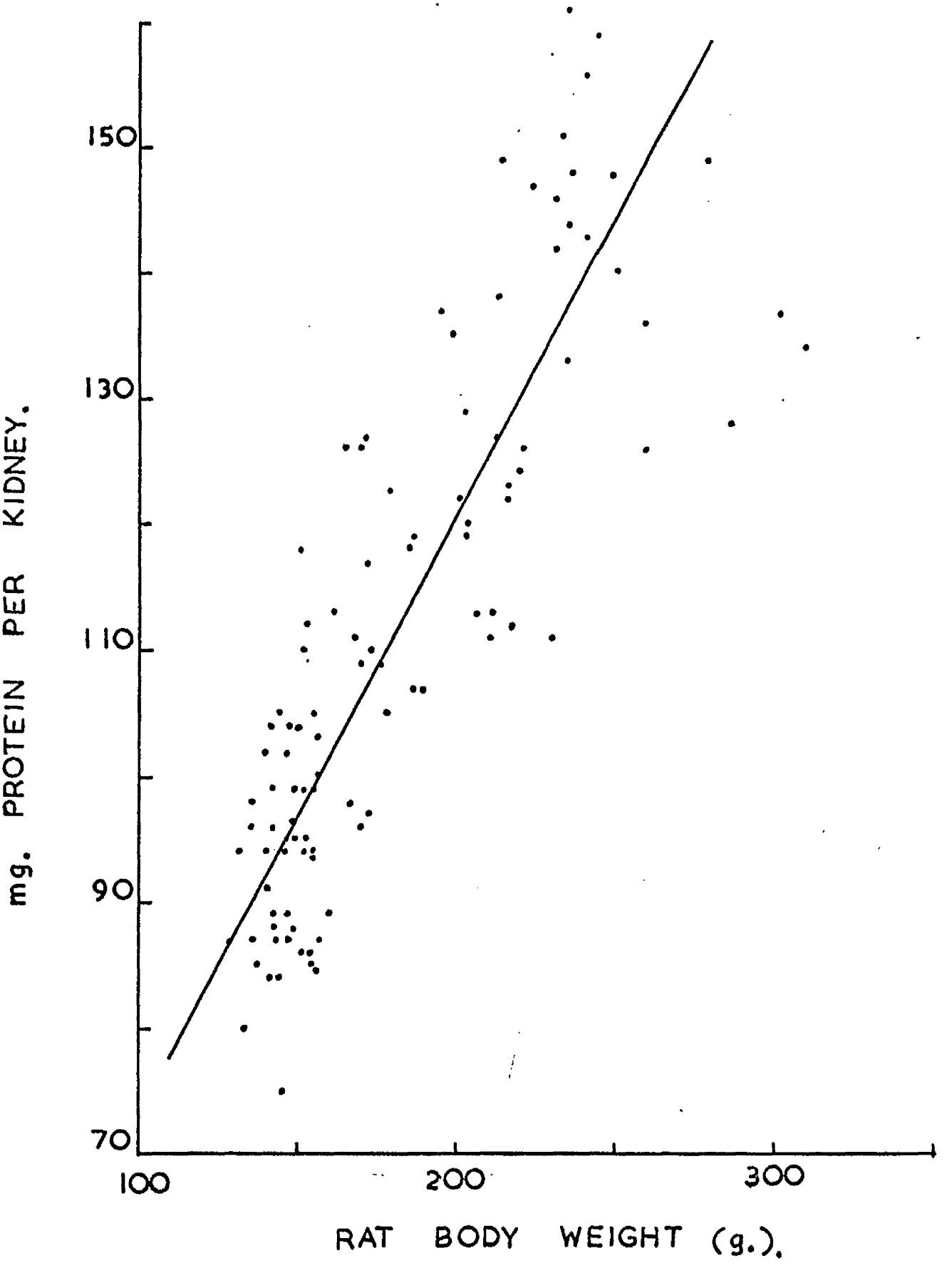

500
400
300
200
100
µg. RNAP PER KIDNEY
100
200
300
RAT BODY WEIGHT (g.)

Figure 15

The correlation between total protein content of the right kidney and body weight of normal male rats.

The equation of the line is $y = 0.475x + 26$ and $r = 0.85$ $(P < 0.001)$.

FIGURE 15.


150
130
110
90
70
mg. PROTEIN PER KIDNEY.
100
200
300
RAT BODY WEIGHT (g.).

body weight on the other. In none of these instances was the correlation coefficient very significant. In other words, these Figures do not reveal any significant relationship between the size of the animal and the size and composition of the cells in its kidneys. The data collected in Figures 16, 17 and 18, however, were derived from control animals used in a variety of experiments carried out over a period of years. If the data in any of these Figures are plotted in such a way as to distinguish between different experiments, as has been done in Figure 16, it becomes immediately clear that the variation within an individual experiment is much less than that in the population at large. This may reflect variations from time to time in kidney composition in the departmental rat colony (from causes unknown), or it may simply indicate a variable error in the methods of estimation. Whichever of these explanations is correct is not very important for the present purpose. What is important is that these data emphasise the necessity of adequate controls within each experiment and the dangers inherent in comparing too confidently absolute figures obtained in two separate experiments. Throughout the present work great care has been exercised both in the design of experiments and in the organisation of analyses to ensure that the results would not be invalidated by the effects of unknown sources of variation in experimental animals or of unknown and variable errors in analytical techniques.

Figure 16

The correlation between DNA concentration of the right kidney and body weight of normal male rats.

The values obtained in different experiments are marked by different symbols.

FIGURE 16.

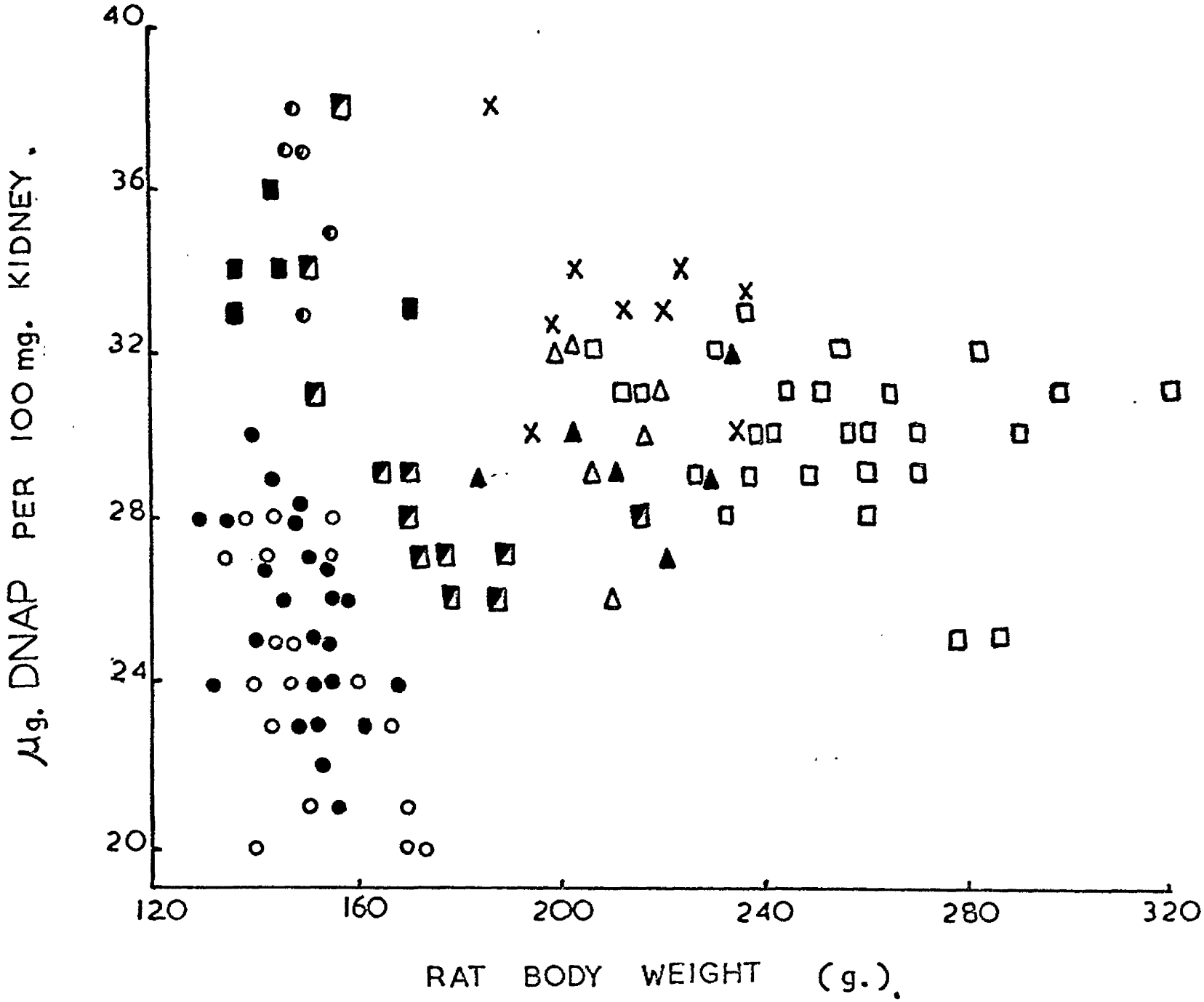

40
36
32
28
24
20
µg. DNAP PER 100 mg. KIDNEY.
120
160
200
240
280
320
RAT BODY WEIGHT (g.).

Figure 17

The correlation between the RNA/DNA ratio of the right kidney and the body weight of normal male rats.

The equation of the line is $y = 0.0006x + 1.343$ and $r = 0.19$ $(P < 0.05)$.

FIGURE 17.

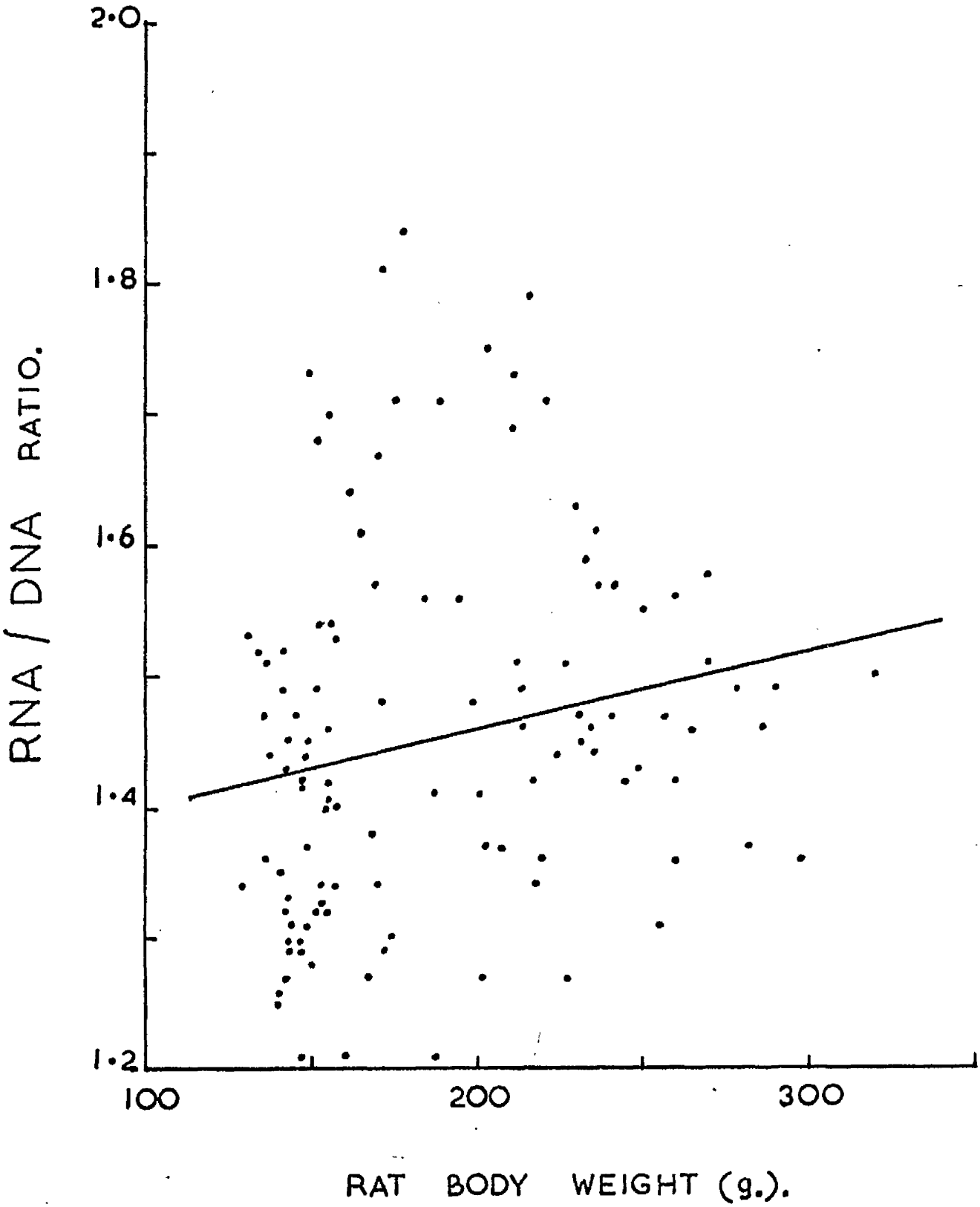
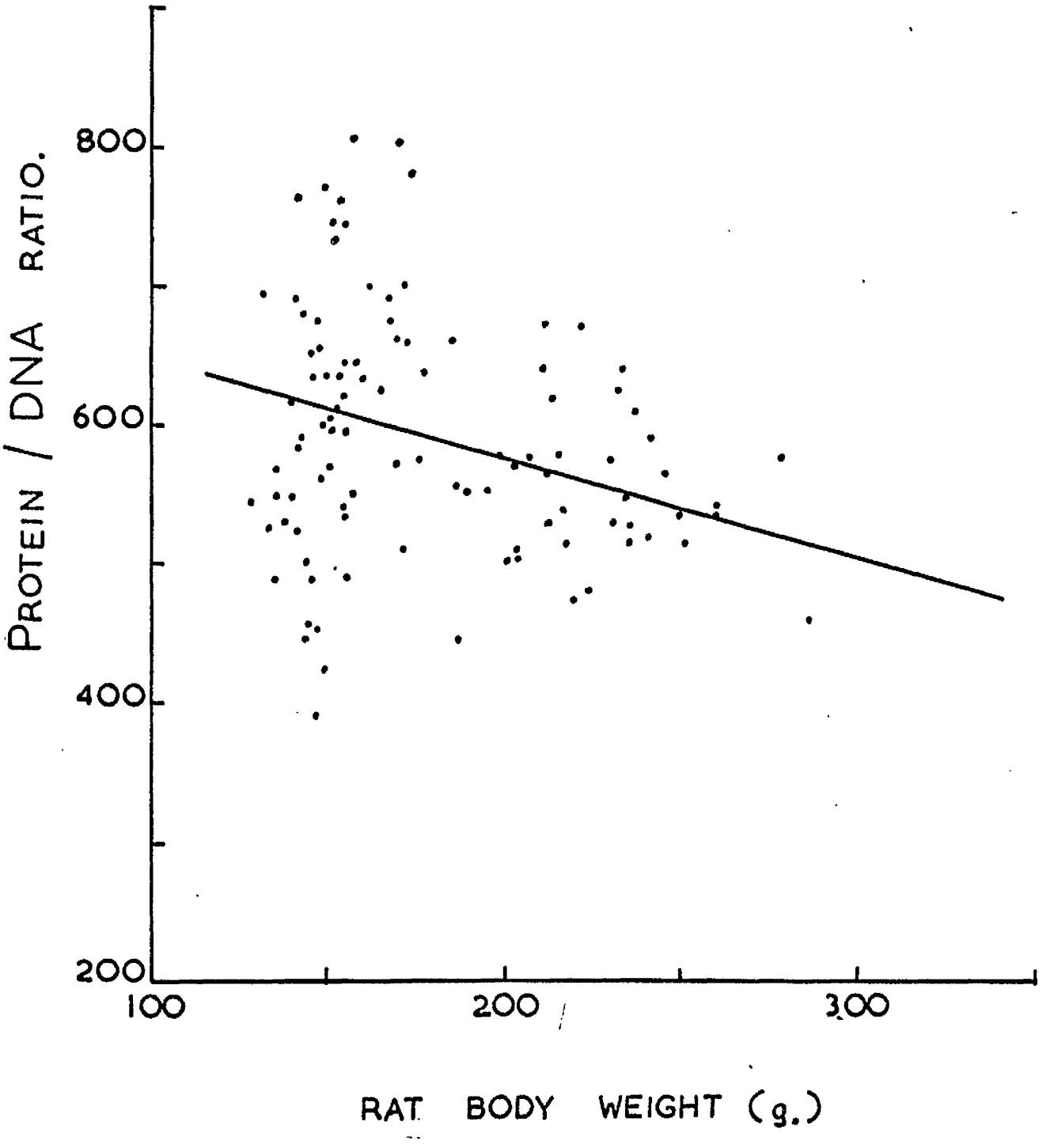


Figure 18

The correlation between the protein/DNA ratio of the right kidney and the body weight of normal male rats.

The equation of the line is $y = -0.695x + 714$ and $r = -0.22$.

FIGURE 18.


PROTEIN / DNA RATIO.
800
600
400
200
100
200
300
RAT BODY WEIGHT (g.)

1.3. Mitotic activity.

In the past, the main alternative to increase in kidney weight as a measurement of compensatory renal hypertrophy has been the counting of mitotic figures in histological sections. Table 14 shows the mitotic frequency in the kidneys of six normal adult rats. In all six animals the frequency was very low, though it appeared to vary a good deal from one individual to another. Because the total number of mitotic figures counted in any one section was so small, the random error attaching to the calculated mitotic frequency is very large. It would, for example, be absurd to claim, on the basis of the figures shown in Table 14, that the mitotic frequency in animal number 1 was significantly different from that in, say, animal number 2 or animal number 3. To obtain significantly more reliable figures it would be necessary to count much larger numbers of mitoses, say a total of 50 per kidney. If we take the mitotic frequency to be the average of the figures shown in Table 14 (i.e. about 1 per 10,000 nuclei), this would necessitate scaning an area equivalent to 500,000 nuclei. At the section thickness we have used this in turn would be equivalent to 40 x 200 = 8,000 fields. This would require a total of approximately 24 hours scanning for each animal. It is clear, therefore, that to obtain a quantitative estimate of mitotic frequency in even a single kidney is likely to be a very laborious undertaking. To obtain an average value for a group of normal animals would be almost prohibitively expensive in

Table 14

Mitotic activity in the kidneys of normal male rats of body weight 220 to 320 g.

Animal No.	Section No.	Estimated No. of Nuclei	Number of Mitoses	Mitoses per 10,000 Nuclei
1	1	14,980	1	1.43
	2	13,730	1	
	3	13,300	4	
2	1	13,930	1	0.52
	2	12,460	0	
	3	12,020	1	
3	1	14,470	4	2.01
	2	11,980	2	
	3	13,330	2	
4	1	10,990	0	0.00
	2	14,120	0	
	3	11,248	0	
5	1	22,160	2	0.70
	2	20,600	1	
6	1	23,540	2	1.69
	2	17,880	5	

200 high-power fields were scanned in each section and the total number of mitotic figures counted. The number of nuclei in every tenth field was counted. The total number of nuclei in these twenty ~~one~~ fields was multiplied by 10 to give the estimated number of nuclei in the 200 fields.

terms of time and labour.

1.4. Summary.

Two main conclusions seem to emerge from the results reported in this section. The first is that the two kidneys of a single animal are remarkably alike. Even though they are not equal in weight, the difference between them is remarkably constant and in composition they seem indistinguishable. Consequently, the progress of compensatory renal hypertrophy can probably be followed satisfactorily by comparing the surviving kidney with the kidney removed at operation. On the other hand, the variation in kidney size and composition between different individuals, even if they are of the same body weight, is quite large. Consequently, if the effect of any factor on kidney size and composition has to be tested by comparison between a treated group of animals and a control group, fairly large numbers of animals will have to be used.

The second main conclusion which can be legitimately drawn is that the labour involved in determining the DNA content of kidneys, in order to estimate cell number, cell size and cell composition, seems to be worthwhile. In the present section it has revealed that the right kidney is larger than the left not because its cells are larger, but because they are more numerous. The variation in kidney size with age and body weight is also a matter of cell number rather than cell size. It would seem reasonable to assume that the same sort of approach is likely to be equally profitable

when applied to the process of compensatory renal hypertrophy. It certainly appears more promising than an approach based on the tedious and rather inaccurate method of counting mitotic figures in histological sections.

2. The effect of unilateral nephrectomy.

Before investigating the mechanism controlling unilateral nephrectomy, it was necessary to define clearly the effect of the operation on the size and composition of the remaining kidney with a view to finding a suitable means of detecting and measuring its growth. The method involved would need to be fairly sensitive since most of the changes described by other workers are relatively small. For this reason it seemed necessary to examine and compare all changes which result from unilateral nephrectomy and to select the one which would provide an easily measurable, but clear and if possible, early indicator of kidney growth. Accordingly the following factors were examined.

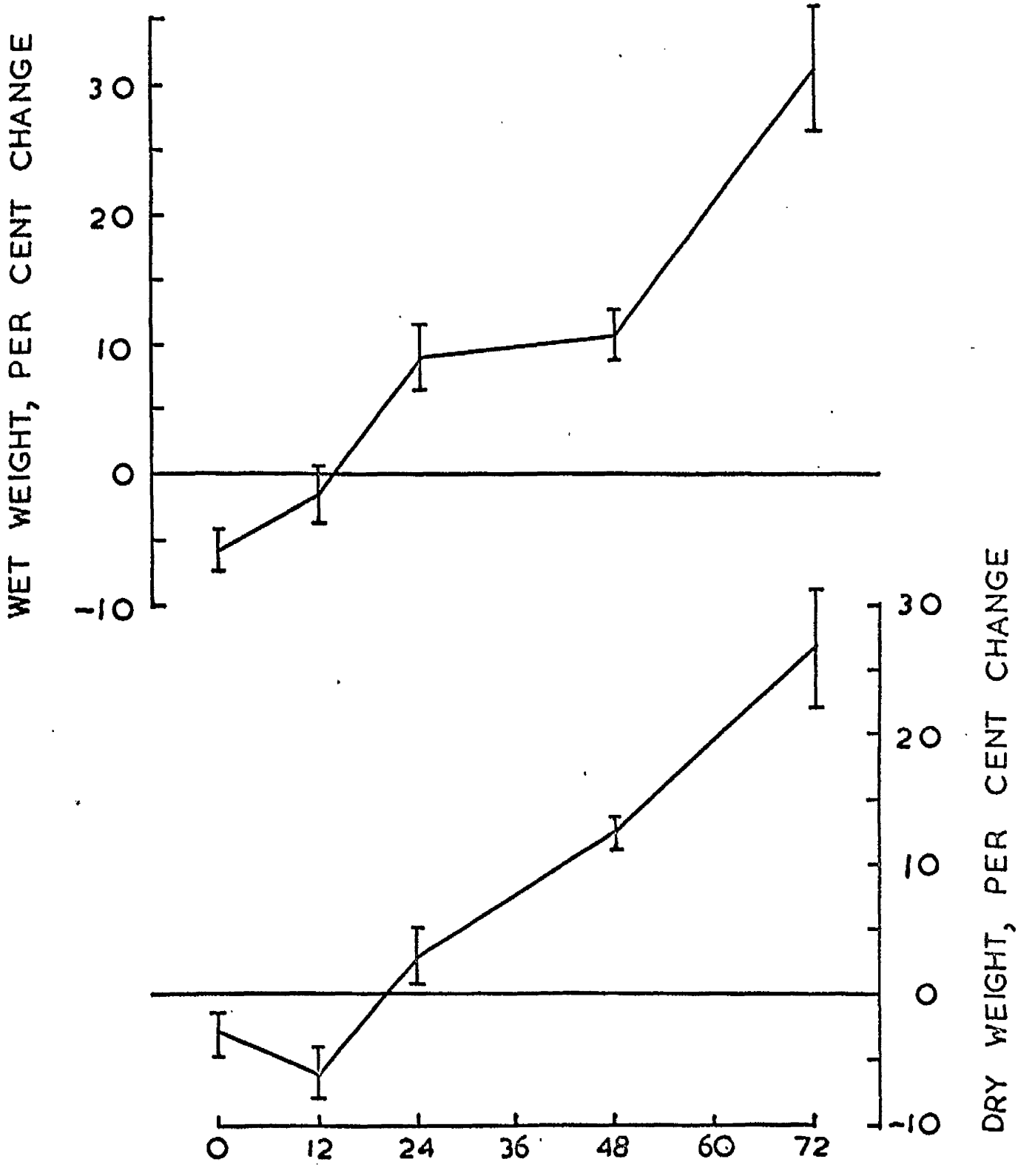
2.1. Changes in kidney weight.

Figure 19 shows the increases in wet and dry weight of the remaining kidney in the first 3 days after unilateral nephrectomy. The results are expressed as the percentage difference between the remaining kidney and the kidney removed at operation. The wet and dry weights of the left kidney, which were initially less than those of the right kidney removed at operation, increased to a value about 30-35% above normal in the 3 day period. The changes in dry weight

Figure 19

The effect of unilateral nephrectomy in rats on the wet and dry weight of the remaining kidney, expressed as a percentage of the weight of the excised kidney.

Each point is the mean for 6 animals; vertical bars represent S.E.M. The animals weighed between 168 and 210 grams.


WET WEIGHT, PER CENT CHANGE
30
20
10
0
-10
DRY WEIGHT, PER CENT CHANGE
30
20
10
0
-10
0
12
24
36
48
60
72
TIME AFTER UNILATERAL
NEPHRECTOMY (HOURS).

appeared to parallel quite closely the changes in wet weight. For purposes of comparison, Figure 20 shows the corresponding information for mice. It is quite clear that in both species the hypertrophy of the remaining kidney is proceeding at roughly the same rate, though in mice the total increase in weight was slightly less. From Figure 19 it is clear that in the rat, the increase in either wet or dry weight is clearly demonstrable at 24 hours but not at 12 hours. It is, however, of interest that the water content of the remaining kidney shows a slight but significant increase detectable both at 12 and at 24 hours after the operation (Table 15). A change as early as this is obviously of interest. Unfortunately in the present instance it is too small to be easy to measure. Moreover, no corresponding change was observed in the mouse experiment (Table 16). It is not therefore likely to be of much help in elucidating the process of compensatory renal hypertrophy. The water content of the surviving kidney after unilateral nephrectomy has also been investigated by Straube and Patt (1961), who used female mice, and by Montfort and Pérez-Tamayo (1962) who used rats. Both groups reported that there was no change in the water content of the kidney remaining after unilateral nephrectomy, but both groups examined the kidneys at time periods which were much longer post-operatively than those employed in the present investigation. Straube and Patt (1961) reported their results at 14 days and Montfort and Pérez-Tamayo (1962) at 10 and 20 days post-operatively. It is possible therefore that the

Figure 20

The effect of unilateral nephrectomy in mice on the wet and dry weight of the remaining kidney, expressed as a percentage of the weight of the excised kidney.

Each point is the mean for 4 animals; vertical bars represent S.E.M. The animals weighed between 24 and 32 grams.

FIGURE 20.

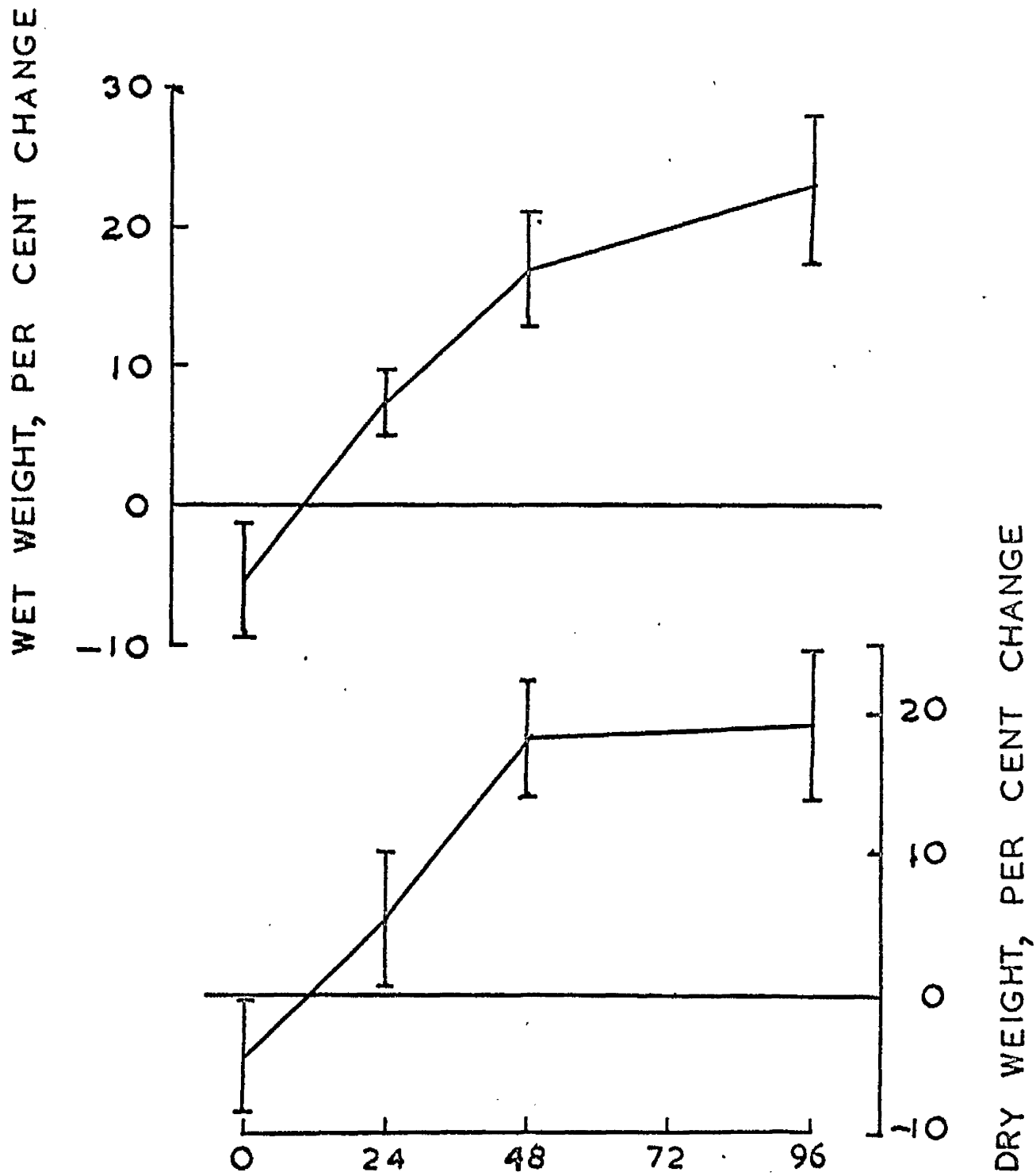

WET WEIGHT, PER CENT CHANGE
30
20
10
0
-10
DRY WEIGHT, PER CENT CHANGE
20
10
0
-10
0
24
48
72
96
TIME AFTER UNILATERAL NEPHRECTOMY (HOURS).

Table 15

Effect of unilateral nephrectomy on the water content of the left kidney of rats.

Time after Unilateral Nephrectomy (Hours)	Water Content (%)		
	Right Kidney	Left Kidney	Difference
0	76.8 ± 0.20	76.1 ± 0.18	-0.67 ± 0.227
12	75.2 ± 0.19	76.2 ± 0.47	1.03 ± 0.420†
24	75.1 ± 0.27	76.4 ± 0.33	1.30 ± 0.223*
48	76.8 ± 0.30	76.2 ± 0.33	-0.45 ± 0.395
72	77.3 ± 0.31	78.0 ± 0.29	0.75 ± 0.304†

Values are means ± S.E.M. for 6 animals weighing between 168 and 210 g.

* Significantly different from the value of the 0 time group with a P value of 0.001 or less.

† Ditto, with a P value of 0.01 or less.

Table 16

Effect of unilateral nephrectomy on the water content of the left kidney of mice.

Time after Unilateral Nephrectomy (Hours)	Water Content (%)		
	Right Kidney (mg.)	Left Kidney (mg.)	Difference in Weight between Right and Left Kidneys. (mg.)
0	74.7 ± 0.50	74.6 ± 0.50	-0.23 ± 0.315
24	74.4 ± 0.30	74.8 ± 1.19	0.38 ± 0.979
48	75.1 ± 0.22	74.8 ± 0.32	-0.30 ± 0.292
96	74.1 ± 0.18	74.9 ± 0.47	0.73 ± 0.406

Values are means ± S.E.M. for 4 animals.

water content of the remaining kidney increases immediately after the operation but returns to normal within a few days. These changes in wet and in dry kidney weight are technically easy to obtain. In addition they give a fairly early indication of gross changes in the surviving kidney after unilateral nephrectomy. Nevertheless, they are not by themselves an adequate measure of kidney growth, since they give no indication of any chemical changes occurring or of the relative contributions of changes in cell number and in cell size. In addition, change in weight is a rather insensitive indicator of growth (because of the large standard errors, see Figures 19 and 20) and it makes no allowance for the blood or urine content of the kidney. In all subsequent studies, however, the wet weight, but not the dry weight, of the kidneys was recorded, since this can be done yet still permit further analysis of the kidneys.

2.2. Changes in mitotic activity.

As has been shown in Section 3.1.3., the mitotic activity of the normal kidney is very low. Table 17 shows the changes in mitotic activity in the surviving kidney in the first 3 days after unilateral nephrectomy. It is quite clear that one day after the operation the mitotic activity was still at the resting level. At 2 days, however, it showed a sharp increase with a further increase at 3 days. In agreement with Goss (1963a), the incidence of mitoses at 48 hours was about 6 times as great as in unoperated controls. There was, however, no apparent peak of mitotic response 40-48 hours post-

Table 17

The frequency of mitoses in the remaining kidneys at various times after right unilateral nephrectomy.

Time After Unilateral Nephrectomy (Days)	Animal No.	Estimated No. of Nuclei	Number of Mitoses	Mitoses per 10,000 Nuclei
0	1	42,010	6	1.43
	2	38,410	2	0.52
	3	39,780	8	2.01
	4	36,360	0	0.00
1	5	37,000	5	1.35
	6	29,590	4	1.35
	7	30,920	4	1.29
	8	32,010	6	1.87
2	9	38,550	12	3.11
	10	36,690	15	4.09
	11	35,210	15	4.26
	12	32,740	34	10.39
3	13	42,350	60	14.17
	14	40,180	40	9.96
	15	39,460	29	7.35
	16	38,190	24	6.28

The animals weighed between 260 and 320 g. Method of counting as in Table 14.

operatively as reported by Ogawa and Sinclair (1958) and by Williams (1961). This discrepancy may perhaps be explained on the assumption that the response to unilateral nephrectomy varies with the age of the animal used (Konishi, 1962). From a practical point of view, the mitotic counts at 2 days and at 3 days after unilateral nephrectomy are sufficiently high to be fairly easy to estimate. Nevertheless, the increase above the control level is not really great enough to make the method a very satisfactory way of following kidney growth. The great advantage of the use of mitotic counts over, say, kidney weight measurements, is that the method provides unequivocal evidence of increase in cell number (as opposed to increase in cell mass). It is perhaps a little surprising that the mitotic increase in compensatory renal hypertrophy should be so small. In many respects, compensatory renal hypertrophy is comparable to the compensatory growth of the remaining liver fragment which follows partial hepatectomy. This latter process is, however, marked by a very much more dramatic mitotic response. In normal liver, mitosis is as infrequent as it is in normal kidney - less than 5 mitoses per 10,000 nuclei (Brues and Marble, 1937; Hammersten, 1951). After partial hepatectomy this figure increases within 48 hours to something like 200 to 300 (Brues and Marble, 1937; Abercrombie and Harkness, 1951; Weinbren, 1959). Why the response of kidney to unilateral nephrectomy should be so much less dramatic is not entirely clear. To some extent it may be explained by the

fact that whereas liver regeneration takes the form of a straightforward increase in cell number, compensatory renal hypertrophy is partly due to increase in cell number and partly to increase in cell size. It is true also that compensatory renal hypertrophy is, on the whole, a slower process than liver regeneration though the difference is not particularly great.

2.3. Changes in chemical composition.

Because compensatory renal hypertrophy involves an increase in both cell number and cell size (Miyada and Kurnick, 1960; Simpson, 1961a; Threlfall et al., 1964), it seemed desirable to obtain a method of following growth of the remaining kidney which gave a measure of both these changes. Table 13 gave a comparison of the chemical composition of the right and left kidneys of normal rats. This investigation gave an indication of the number of cells in the kidneys in terms of their DNA content and an indication of the average composition of the cells was obtained by relating other cell constituents to DNA. These results should serve as a basis for an investigation of the chemical changes in the surviving kidney after unilateral nephrectomy. Table 18 shows the results of an experiment in which the right kidney (i.e. the larger of the two) was removed. Forty eight hours later the remaining kidney had increased significantly in size ($P < 0.001$) as might have been expected from previous experience (Figure 19). This was accompanied by a small but significant increase in DNA per kidney ($P < 0.02$) i.e.

Table 18

Effect of right unilateral nephrectomy on the weight and nucleic acid content of the left kidney after 48 hours.

	Right Kidney	Left Kidney	Ratio $\frac{\text{Left}}{\text{Right}}$
Weight (mg.)	800 ± 23.7	838 ± 27.3	1.05 ± 0.017*
DNAP			
µg/100 mg. kidney	33.2 ± 1.88	33.1 ± 1.39	1.00 ± 0.032
µg/kidney	244 ± 7.6	247 ± 7.1	1.01 ± 0.011†
RNAP			
µg/kidney	347 ± 19.6	462 ± 25.4	1.33 ± 0.032*
µg/µg. DNAP	1.42 ± 0.05	1.87 ± 0.06	1.32 ± 0.031*

Values are means ± S.E.M. for 6 animals weighing between 230 and 270 g.

* Ratio significantly different from the corresponding ratio for the unoperated control rats (Table 13) with a P value of 0.001 or less.

†Ditto, with a P value of 0.02 or less.

in cell number, but not by any change in mean cell mass as reflected in DNA concentration per 100 mg. tissue. On the other hand, there had been a very large increase in RNA/DNA ratio (i.e. RNA per cell). Table 19 shows the results of a corresponding experiment in which the animals were sacrificed 96 hours after the operation. At this time interval there had been a further increase in kidney weight and a corresponding increase in cell number. The mean cell mass at this time had also increased, but only slightly. In this experiment protein was included in the analysis, but the small apparent increase in protein content per cell was not statistically significant. The RNA content per cell, on the other hand, was still at the same high level as it had been in the 48 hour experiment. Putting together the results of the two experiments (Tables 18 and 19), the picture one obtains is of a rather slow but quite unmistakeable increase in cell number accompanied by, or perhaps followed by, a small increase in cell mass. Far more dramatic than either is the large increase in RNA/DNA ratio.

The end result of compensatory renal hypertrophy is of course an increase in both cell number and cell size. The results in Tables 18 and 19 seem to suggest that the increase in cell number might come first. This point required confirmation. Accordingly a second 48 hour experiment was carried out in which protein estimations (which had been omitted from the experiment in Table 18) were included. As a check on the determination, protein was

Table 19

Effect of right unilateral nephrectomy on the weight and nucleic acid content of the left kidney after 96 hours.

	Right Kidney	Left Kidney	Ratio $\frac{\text{Left}}{\text{Right}}$
Weight (mg.)	857 ± 18.4	949 ± 32.1	1.11 ± 0.018*
DNAP			
µg/100 mg. kidney	30.0 ± 0.56	28.2 ± 0.63	0.94 ± 0.018†
µg/kidney	257 ± 7.2	267 ± 8.7	1.04 ± 0.013†
RNAP			
µg/kidney	366 ± 14.1	512 ± 15.9	1.40 ± 0.100*
µg/µg. DNAP	1.49 ± 0.02	1.92 ± 0.04	1.29 ± 0.013*
Protein			
mg./kidney	152 ± 1.8	166 ± 4.4	1.09 ± 0.030‡
µg/µg. DNAP	592 ± 14.5	622 ± 10.7	1.05 ± 0.022

Values are means ± S.E.M. for 6 animals weighing between 220 and 245 g.

* Ratio significantly different from the corresponding ratio for the unoperated control rats (Table 13) with a P value of 0.001 or less.

† Ditto, with a P value of 0.01 or less.

‡ Ditto, with a P value of 0.05 or less.

Table 20

The effect of right unilateral nephrectomy on the weight, nucleic acid content, total protein and protein nitrogen content of the left kidney after 48 hours.

	Right Kidney	Left Kidney	Ratio $\frac{\text{Left}}{\text{Right}}$
Weight (mg.)	716 ± 14.8	797 ± 26.1	1.11 ± 0.024*
DNAP			
μg/100 mg. kidney	30.1 ± 0.88	24.2 ± 0.80	0.81 ± 0.022*
μg/kidney	216 ± 10.0	194 ± 9.5	0.90 ± 0.026
RNAP			
μg/kidney	304 ± 8.7	379 ± 14.0	1.25 ± 0.029*
μg/μg. DNAP	1.42 ± 0.05	1.98 ± 0.06	1.39 ± 0.037*
Protein (Lowry method)			
mg/kidney	117 ± 2.2	129 ± 4.3	1.10 ± 0.028†
μg/μg. DNAP	546 ± 19.8	676 ± 26.9	1.24 ± 0.032*
Protein Nitrogen (micro-Kjeldahl method)			
mg/kidney	17.6 ± 6.0	19.6 ± 5.3	1.11 ± 0.030
μg/μg. DNAP	82 ± 2.5	103 ± 4.7	1.26 ± 0.048

Values are means ± S.E.M. for 6 male rats weighing between 201 and 218 g.

* Significantly different from the corresponding ratio for the unoperated control animals (Table 13) with a P value of 0.001 or less.

† Ditto, with a P value of 0.01 or less.

estimated by two methods, the biuret method of Lowry et al. (1951) and the micro-Kjeldahl method. As can be seen from Table 20, they gave concordant results. Since, however, the method of Lowry et al. (1951) was simpler and quicker, it was used in all subsequent estimations. The results of this experiment differed in several important particulars from those obtained in the earlier 48 hour and 96 hour experiments. The points of difference are summarised in Table 21. In all three experiments there was a comparable increase in kidney weight. In the earlier experiments this seemed to be attributable to roughly equivalent increases in cell number and in cell size (last two columns of Table 21). In the new experiment however (first column of Table 21), there was no increase in cell number and the increase in cell size was much larger. The changes in protein content per cell were very much in agreement with the changes in cell size, but in all three experiments, the RNA content per cell showed the same 30 to 40% increase over the control figure.

The apparent contradiction between Tables 18 and 20 (brought out in Table 21) was resolved when similar experiments were carried out at shorter time intervals. The results of these are shown in Tables 22 and 23. It should be noted that in these experiments half the animals were subjected to right unilateral nephrectomy and half to left. The object of this was to balance out the difference in size and cell number between the right and left kidney before the operation and thus to obviate the necessity of comparing the analyses

Table 21

Comparison of the changes in the remaining kidney at 48 and 96 hours after unilateral nephrectomy.

	48 Hours		96 Hours
	Table 20	Table 18	Table 19
Body Weight	210 g.	251 g.	234 g.
Weight of Kidney Excised at Operation	716 mg.	800 mg.	857 mg.
Increase in Kidney Weight	21%*	15%*	21%*
Increase in Cell Number	- 4%	7%‡	10%†
Increase in Cell Size	23%*	4%	10%†
Increase in Protein per Cell	25%*	-	6%
Increase in RNA per Cell	39%*	32%*	29%*

* Significant with a P value of 0.001 or less.

† Significant with a P value of 0.01 or less.

‡ Significant with a P value of 0.02 or less.

Table 22

The effect of unilateral nephrectomy on the nucleic acid content of the surviving kidney after 24 hours.

	Excised Kidney	Surviving Kidney	Ratio $\frac{\text{Surviving}}{\text{Excised}}$
DNAP			
µg/100 mg. kidney	31.3 ± 0.34	26.1 ± 1.21	0.84 ± 0.044 ‡
µg/kidney	291 ± 11.6	291 ± 10.5	1.00 ± 0.025
RNAP			
µg/kidney	437 ± 17.5	549 ± 35.5	1.26 ± 0.043 †
µg/µg DNAP	1.50 ± 0.04	1.88 ± 0.09	1.25 ± 0.031*

Values are means ± S.E.M. for 6 animals weighing between 250 and 300 g. In order to balance out the differences between right and left kidneys before the operation, 3 animals were subjected to right unilateral nephrectomy and 3 to left unilateral nephrectomy. The ratios in the column on the extreme right were therefore compared with unity instead of with the corresponding ratios for the unoperated control animals (Table 13).

* Ratio significantly different from unity with a P value of 0.001 or less.

† Ditto, with a P value of 0.01 or less.

‡ Ditto, with a P value of 0.02 or less.

Table 23

The effect of unilateral nephrectomy on the nucleic acid and protein content of the surviving kidney after 12 hours.

	Excised Kidney	Surviving Kidney	Ratio $\frac{\text{Surviving}}{\text{Excised}}$
DNAP			
μg/100 mg. kidney	30.9 ± 0.63	30.4 ± 0.41	0.99 ± 0.026
μg/kidney	236 ± 11.1	225 ± 12.4	0.95 ± 0.015
RNAP			
μg/kidney	345 ± 19.2	349 ± 19.4	1.01 ± 0.020
μg/μg DNAP	1.46 ± 0.03	1.56 ± 0.02	1.07 ± 0.022*
Protein			
mg/kidney	123 ± 7.6	119 ± 6.9	0.97 ± 0.027
μg/μg DNAP	523 ± 12.7	528 ± 15.3	1.01 ± 0.021

Values are means ± S.E.M. for 6 animals weighing between 215 and 260 g. In order to balance out the differences between right and left kidneys before the operation, 3 animals were subjected to right unilateral nephrectomy and 3 to left unilateral nephrectomy. The ratios in the column on the extreme right were therefore compared with unity instead of with the corresponding ratios for the unoperated control animals (Table 13).

* Ratio significantly different from unity with a P value of 0.02 or less.

with the corresponding figures for the control group in Table 13. Although the complete results for these experiments are shown in Tables 22 and 23, in order to facilitate comparison with other experiments, the salient points are summarised in Table 24. As this Table shows, the changes in the surviving kidney 24 hours after unilateral nephrectomy are similar to those found in the second 48 hour experiment (Table 20). That is to say, the kidney has increased in weight by about 20%, but this is entirely accounted for by an increase in cell size. There has been no increase whatever in cell number. Once again the most dramatic change has been the increase in RNA/DNA ratio. The changes in the surviving kidney twelve hours after unilateral nephrectomy are very slight. At this stage there has been no increase in organ weight, or in cell number, or in cell size, or in the average protein content per cell. The sole significant change is a 7% increase in RNA per cell.

We are now in a position to try to construct a consistent picture from these experiments. It would seem to be as follows. In the first twelve hours after unilateral nephrectomy, the surviving kidney does not grow although there is some accumulation of RNA, presumably in preparation for growth. Between 12 and 24 hours, the kidney increases substantially in size, the increase being attributable to enlargement of the cells rather than to cell division. This phase appears to continue until about 48 hours after the operation. The 48 hour results in Table 20 show essentially the same pattern as

Table 24

Comparison of the changes in the remaining kidney at 12 and 24 hours after unilateral nephrectomy.

	12 Hours	24 Hours
	Table 23	Table 22
Body Weight	235 g.	288 g.
Weight of Kidney Excised at Operation	771 mg.	931 mg.
Increase in Kidney Weight	-4%	22%*
Increase in Cell Number	-	0
Increase in Cell Size	1%	16%*
Increase in Protein per Cell	1%	-
Increase in RNA per Cell	7%†	25%*

* Significantly different from zero with a P value of 0.001 or less.

† Ditto, with a P value of 0.01 or less.

the 24 hour results in Table 22. About 48 hours after the operation, however, cell number begins to increase and at the same time the cells diminish somewhat in size, so that at this stage the overall increase in organ size is due to roughly equivalent increases in cell number and cell size. This stage is shown very clearly in the 96 hour experiment (Table 19) but the same pattern is discernable in the earlier of the 48 hour experiments (Table 18). Thus 48 hours appears to mark approximately the point at which cell division gets under way. The time scale of the process probably depends on circumstances. In the first 48 hour experiment (Table 18) cell division had already started. In the second 48 hour experiment (Table 20) it had not yet begun. Regardless of cell growth and cell division however, the most striking and consistent feature of the whole process was an increase in RNA/DNA ratio, detectable at 12 hours when no other changes were apparent, reaching 25% at 24 hours and remaining at or above this level at 48 and 96 hours.

2.4. Changes in enzymes.

The changes in cell number and cell composition, particularly the changes in RNA/DNA ratio, appear to offer a satisfactory method of measuring the growth of the kidney remaining after unilateral nephrectomy. These chemical changes, however, are more an indication of growth than one of its causes and must themselves be brought about by other factors. Ultimately a study must be made of the enzymes producing these changes, where the controlling mechanisms are likely

to act. Accordingly, three enzymes which have previously been shown by other workers to be associated with growth were examined, in the hope that their increased activity might offer an even earlier indication of growth.

The first of these enzymes to be examined was DNA deoxynucleotidyltransferase, which is involved in one of the final steps in the production of DNA. Although DNA synthesis does occur in the surviving kidney after unilateral nephrectomy, it has been shown in the preceding section that an increase in total DNA content of the remaining kidney cannot be detected with any certainty before 2-4 days postoperatively. It seemed likely that the activity of DNA deoxynucleotidyltransferase would show an increase at an earlier time. The activity of this enzyme was therefore examined in the kidneys remaining after unilateral nephrectomy and compared with the kidneys removed at operation. Table 25 shows that there was no clear increase in the activity of this enzyme in the remaining kidney. It is possible that the specific activity of the enzyme increased, but since only gross changes were being sought, for convenience the results were expressed somewhat crudely in terms of tissue weight rather than in terms of protein. This assay, therefore, does not provide a suitable means of following the growth of the remaining kidney.

The possibility of an increase in DNA deoxynucleotidyltransferase activity was consistent with the process of growth. Increases in

Table 25

DNA deoxynucleotidyltransferase activity before and 48 hours after right unilateral nephrectomy.

Kidney	Time of Removal (Hours)	Kidney Weight (mg.)	Enzyme Activity counts/min.	
			Per 100 mg. Wet Weight Kidney	Per Kidney
Right	0	768	167	1447
Left	48	865	170	1516
Right	0	890	169	1211
Left	48	916	76	544
Right	0	718	165	1424
Left	48	824	130	1193
Right	0	718	124	1017
Left	48	844	201	1692

Animals weighed between 205 and 212 g.

The enzyme activities were determined as described in section 2.3.1.

Table 26

Deoxyribonuclease II assay before and 48 hours after right unilateral nephrectomy.

Kidney	Time of Removal (Hours)	Kidney Weight (mg.)	Enzyme Activity Extinction at 260 mμ.	
			Per 100 mg. Wet Weight Kidney	Per Kidney
Right	0	788	5.2	41
Left	48	908	4.7	43
Right	0	690	4.9	34
Left	48	835	5.2	43
Right	0	693	4.7	33
Left	48	999	5.5	54
Right	0	755	5.4	40
Left	48	880	5.9	52

The animals weighed between 209 and 217 g.

The enzyme activities were determined as described in section 2.3.3.

Table 27

Deoxyribonuclease I assay before and 48 hours after right unilateral nephrectomy.

Kidney	Time of Removal (Hours)	Kidney Weight (mg.)	Enzyme Activity Extinction at 260 mμ.	
			Per 100 mg. Wet Weight Kidney	Per Kidney
Right	0	660	14.1	93
Left	48	805	16.2	130
Right	0	720	11.1	80
Left	48	865	11.0	95

The animals weighed between 207 and 210 g.

The enzyme activities were determined as described in section 2.3.2.

ribonuclease and deoxyribonuclease activities have, however, also been noted in some non-malignant growing tissues such as placenta, bone marrow, normal liver and regenerating liver (Brody, 1953, 1958; Brody and Thorell, 1957; Adams, 1963). The significance of these changes is not clear but since these enzymes have been shown to increase in activity in so many growing mammalian tissues, the activity of two of them, deoxyribonuclease I and deoxyribonuclease II was compared before and 48 hours after unilateral nephrectomy. Table 26 shows that deoxyribonuclease II showed a slight increase in activity in the remaining kidney at this time. Table 27 shows that the activity of deoxyribonuclease I also increased slightly in the same time. Since these changes are difficult to explain, however, and since none of the enzyme activities studied altered very markedly, they were not regarded as providing a convenient method of following the growth of the remaining kidney.

2.5. Serum electrolytes and blood haematocrit.

The earliest changes of any kind reported after unilateral nephrectomy have been an increase in the excretion of water and of sodium ions by the remaining kidney (Peters, 1963). These changes began during the first hour post-operatively. Such rapid changes could conceivably be involved in the stimulus to compensatory growth of the remaining kidney. Accordingly, serum electrolyte levels were examined after unilateral nephrectomy. Table 28 shows the results obtained in unilaterally nephrectomized and sham operated animals.

Table 28

The effect of unilateral nephrectomy on serum electrolytes and haematocrits.

Time After Unilateral Nephrectomy (Hours)	Sodium (m equiv./l)		Potassium (m equiv./l)		Chloride (m equiv./l)		Haematocrit (%)	
	Sham Operated	U. N.*	Sham Operated	U. N.*	Sham Operated	U. N.*	Sham Operated	U. N.*
Unoperated Controls	149	150	3.9	4.6	106	104	45	45
24	154	151	3.2	3.6	103	107	44	43
48	150	150	3.6	3.4	106	106	39	41

Values are the means of two determinations for animals weighing between 200 and 230 g.

* U. N. stands for unilaterally nephrectomized animals.

There was no consistent change in serum sodium, potassium, chloride or blood haematocrit levels at 24 or 48 hours after unilateral nephrectomy. Brunner, Kuschinsky and Peters (1959) obtained similar results with plasma sodium and blood haematocrits but found a fall in plasma chloride 24, 48 and 96 hours post-operatively and a rise in plasma potassium at the same time intervals. There is therefore some uncertainty about the electrolyte levels at longer time intervals after unilateral nephrectomy and even if changes do occur within an hour or two of the operation (as claimed by Peters), the way in which electrolytes might stimulate nucleic acid and protein synthesis remains to be determined.

2.6. Summary.

After unilateral nephrectomy, the surviving kidney increases in wet and in dry weight. This is accompanied by a much slower increase in total DNA content and in mitotic activity, although these changes cannot be demonstrated by increases in the activity of DNA deoxynucleotidyltransferase. There is, in addition, a small increase in protein/DNA ratio and a dramatic increase in RNA/DNA ratio. Although there is an increase in deoxyribonuclease I and II activities post-operatively, the changes occurring are too small to be of practical value. No changes in serum electrolytes or in blood haematocrits were found. Of these changes, the increases in chemical composition, particularly the increase in RNA/DNA ratio, clearly offer the best method for a precise quantitative measure of

the changes in growth of the remaining kidney.

3. The "work hypertrophy" theory.

From the review of the literature (Section 1.4.) it will be clear that the majority of authors who have tried to explain compensatory renal hypertrophy have favoured the idea that it is a response to a situation in which one kidney has to cope with the work normally shared between two. The assumption implicit in this view is that the size of the kidneys is determined, at least in part, by the amount of work they have to perform. If this is so, it should be possible to produce growth of the kidneys in intact animals by increasing their work load. Many attempts have been made to subject this prediction to experimental test. One type of experiment, which is very attractive in theory, involves severing the ureter and either leaving its proximal end to drain into the peritoneal cavity, or alternatively, implanting it in the duodenum. In either case, the intention is that the urine passing along the ureter should be re-absorbed into the blood stream. In such a preparation therefore, though both kidneys will be equally healthy, the output of only one will be actually excreted. The two kidneys will therefore have to work at twice the normal rate to clear the normal excretory load. Although there is some disagreement about the effect of this procedure on the size of the kidneys, most workers have found very little effect. Technical difficulties may provide an explanation of these rather disappointing results. While

attractive in theory, in practice these surgical procedures do not always produce the desired effects. For example urine discharged into the peritoneal cavity is not, in general, completely reabsorbed (Simpson, 1961a) and in any case, its presence leads to inflammation which appears to have side effects on the kidney (Royce, 1963). It is also possible that the severed ureter may become ~~inflamed~~ INFLAMED or blocked. Such an experiment has, therefore, doubtful significance. An alternative way of increasing the work the kidneys must do is to give, in the diet, an excess of material which must be excreted via the kidneys. This type of experiment seems less liable to stress the animals. Compared with the surgical type of experiment it has, however, the disadvantage that it does not test all the excretory functions of the kidney simultaneously. The effect of a number of dietary factors on kidney size and composition was therefore examined and compared with the effect of unilateral nephrectomy on the remaining kidney.

3.1. The effect of diets high in protein.

It has been shown by a number of workers that diets high in protein produce kidney hypertrophy (Section 1.4.1.). To test whether the effects of a high-protein diet resemble those of unilateral nephrectomy which were described above (Section 3.2.3.), an experiment was set up as follows. Twenty four rats weighing 170-210 g. were divided randomly into two groups; one group was fed a high-protein diet containing twice as much protein as the normal

stock diet, and the other was fed a protein-free diet. After 4 days, half the animals in each group were subjected to right unilateral nephrectomy and the remainder to a sham operation. After a further 4 days on the same diets, all the animals were killed and their kidneys analysed. The feeding of the protein-free diet resulted in an average loss of 12 g. in body weight over the first 4 days and a further 10 g. loss in the second 4. The body weights of the rats receiving the high-protein diet remained steady over the period of the experiment. These findings are in agreement with those of other workers (Thomson et al., 1953; Fleck and Munro, 1963).

Table 29 shows the effects of diet and of right unilateral nephrectomy on the size and composition of the left kidneys. Both in the nephrectomized and sham-operated groups the kidney weights were significantly greater in animals fed the high-protein diet than in those fed the protein-free diet ($P < 0.001$). There was, however, no significant difference in the total DNA content i.e. in cell number. In each group, the animals on the high-protein diet had significantly larger amounts of RNA ($P < 0.01$), protein ($P < 0.001$) and lipid phosphorus ($P < 0.01$) per kidney. Similarly, whether the animals were on the high-protein or protein-free diet, unilateral nephrectomy increased the kidney weight ($P < 0.001$) but not the cell number as reflected in the total content of DNA. The total contents of RNA ($P < 0.01$), protein ($P < 0.01$) and lipid phosphorus ($P < 0.001$) per kidney were also increased after the operation. Table 30 shows

Table 29

The effect of diet and right unilateral nephrectomy on the size and composition of the left kidney.

Diet	Treatment	Rat Body Weight (g.)	Kidney Weight (mg.)	DNAP µg/kidney	RNAP µg/kidney	Protein mg/kidney	Lipid Phosphorus µg/kidney
Protein-Free	Sham Operation	191 ± 4.9	634 ± 12.6	238 ± 8.8	325 ± 27.2	137 ± 5.0	686 ± 81.9
High-Protein	Sham Operation	187 ± 5.2	748 ± 27.4	251 ± 11.9	395 ± 16.1	163 ± 6.4	910 ± 36.3
Protein-Free	Unilateral Nephrectomy	191 ± 5.9	745 ± 28.0	257 ± 10.2	403 ± 12.0	155 ± 6.5	1052 ± 69.7
High-Protein	Unilateral Nephrectomy	193 ± 6.5	959 ± 46.7	263 ± 12.7	529 ± 43.3	215 ± 13.8	1323 ± 95.9

The values are the means ± S.E.M. for six animals.

the effects of diet and of right unilateral nephrectomy on the mean cell size and composition per cell of the left kidney. As reported above, there was no significant difference in the total DNA content of the kidneys as the result of diet, but the DNA concentrations in the animals fed the high-protein diet were significantly less than those in the animals fed the protein-free diets ($P < 0.001$). This probably means that the mean cell mass of the animals fed the high-protein diets was increasing in preparation for cell division. Apparently, therefore, the diet had affected cell size but not cell number. In each group the animals on the high-protein diet had significantly larger RNA/DNA ($P < 0.001$), protein/DNA ($P < 0.01$) and lipid phosphorus/DNA ($P < 0.025$) ratios. Similarly, regardless of the diet fed, unilateral nephrectomy decreased the DNA concentration ($P < 0.01$) and thus increased the mean cell mass. The RNA/DNA ($P < 0.001$), protein/DNA ($P < 0.05$) and lipid phosphorus/DNA ($P < 0.001$) ratios were also increased after the operation. The effects of both diet and operation on kidney size and composition, therefore, do not appear to be due to changes in cell number. This may seem surprising in view of the fact that previous experiments (Table 21) indicated a 10% increase in cell number in the surviving kidney 4 days after unilateral nephrectomy. Some caution is however necessary here. In the experiments described in the previous section, kidneys surviving after unilateral nephrectomy were compared with their partners removed at operation. As has been explained in Section 3.2.,

Table 30

The effect of diet and right unilateral nephrectomy on the mean cell size and composition of the left kidney.

Diet	Treatment	Rat Body Weight (g.)	DNAP µg/100 mg. kidney	RNAP µg/µg. DNAP	Protein µg/µg. DNAP	Lipid Phosphorus µg/µg. DNAP
Protein-Free	Sham Operation	191 ± 4.9	38.2 ± 1.98	1.36 ± 0.081	579 ± 34.8	2.9 ± 0.30
High-Protein	Sham Operation	187 ± 5.2	33.4 ± 0.65	1.59 ± 0.062	657 ± 13.6	3.7 ± 0.13
Protein-Free	Unilateral Nephrectomy	191 ± 5.9	34.6 ± 0.84	1.58 ± 0.061	609 ± 36.5	4.2 ± 0.33
High-Protein	Unilateral Nephrectomy	193 ± 6.5	27.5 ± 1.15	2.01 ± 0.091	815 ± 25.1	5.1 ± 0.29

The values are the means ± S.E.M. for six animals.

the close similarity between the two kidneys in the same animal makes this sort of comparison a very sensitive means of detecting changes in the surviving kidney. The present experimental design, in which kidneys of different individuals are compared, is much less sensitive.

Irrespective of whether either diet or operation may have produced a small and undetected increase in cell number in the present instance, there can be no doubt of their effects on cell size and cell composition. Both in the nephrectomized and sham operated groups the animals on high-protein diet had significantly larger cells than those on the protein-free diet, with more RNA, protein and lipid phosphorus. Similarly regardless of diet, the nephrectomized groups also had larger cells than the sham operated, again with more RNA, protein and lipid phosphorus. This is of course the same sort of pattern as seen in previous nephrectomy experiments (Section 3.2.3.). In the present experiment, the most striking feature was that, very roughly, the effects of diet were of the same magnitude as the effects of operation. Moreover, the two effects seem to be independent of each other and approximately additive. Konishi (1962), using mitotic counts as an index of kidney growth and Reid (1944) and Francis, Smith and Moise (1931), using kidney weight, have also found that the effects of unilateral nephrectomy and of high dietary protein are additive.

Table 31 shows the size and composition of the right kidneys

Table 31

The effect of diet and sham operation on the size and composition of the right kidney.

Diet	Treatment	Rat Body Weight (g.)	Kidney Weight (mg.)	DNAP µg/kidney	RNAP µg/kidney	Protein mg/kidney	Lipid Phosphorus µg/kidney
Protein-Free	4 Days	191 ± 5.9	645 ± 14.2	233 ± 15.5	289 ± 10.4	148 ± 1.8	844 ± 64.3
	8 days + Sham Operation	191 ± 4.9	685 ± 28.0	243 ± 7.1	355 ± 25.2	141 ± 5.1	846 ± 60.2
High-Protein	4 Days	193 ± 6.5	771 ± 44.2	228 ± 18.2	349 ± 17.9	176 ± 9.6	919 ± 85.8
	8 days + Sham Operation	187 ± 5.2	865 ± 37.2	248 ± 17.5	423 ± 19.6	182 ± 4.9	917 ± 64.5

The values are the means ± S.E.M. for six animals.

removed at the unilateral nephrectomy (i.e. after 4 days on the diets) and of the right kidneys of the sham operated animals removed after 8 days. The different diets gave differences in kidney weight ($P < 0.001$), total content of RNA ($P < 0.01$) and protein ($P < 0.001$) but no change in total content of lipid phosphorus or DNA. Table 32 shows that the diets gave differences also in DNA concentration ($P < 0.001$) and in RNA content per cell ($P < 0.001$) and protein content per cell ($P < 0.01$). These results were the same as the effects of diet on the left kidney except for the lipid phosphorus values. Although there was an apparent increase in lipid phosphorus values in the right kidney, the changes were not statistically significant due to a large scatter in values within each group.

Comparison of the right kidneys removed at operation with those removed from the sham operated animals at death (Tables 31 and 32) showed no significant differences except in total RNA content, which was higher in the latter than in the former ($P < 0.01$). Although it is reasonable to find such an increase in animals fed a high-protein diet, it is surprising to find the RNA content of kidney cells increasing in animals fed a protein-free diet. One possible explanation is that the tissue damage effecting during the sham operation has produced some stimulus to growth. Apart from this it would appear that the changes in kidney composition brought about by the changes in diet are complete in the first 4 days and that there is no significant change in the following 4 days. It should

Table 32

The effect of diet and right unilateral nephrectomy on the mean cell size and composition of the right kidney.

Diet	Treatment	Rat Body Weight (g.)	DNAP µg/100 mg. kidney	RNAP µg/µg. DNAP	Protein µg/µg. DNAP	Lipid Phosphorus µg/µg. DNAP
Protein-Free	4 days	191 ± 5.9	35.9 ± 1.88	1.25 ± 0.081	651 ± 44.0	3.7 ± 0.36
	8 days + Sham Operation	191 ± 4.9	35.6 ± 0.91	1.37 ± 0.076	581 ± 21.7	3.5 ± 0.16
High-Protein	4 days	193 ± 6.5	29.6 ± 1.45	1.55 ± 0.073	787 ± 42.6	4.1 ± 0.22
	8 days + Sham Operation	187 ± 5.2	28.6 ± 0.97	1.72 ± 0.057	750 ± 43.5	3.8 ± 0.09

The values are means ± S.E.M. for six animals.

be pointed out that since the right kidneys were not analysed at the same time as the left kidneys, the results for the two kidneys, even of the same animal, cannot be compared too closely due to possible variation in analyses.

It is interesting to note that the protein-free diet depressed the RNA/DNA ratio to 1.25-1.37 (Tables 30 and 32) compared to the figure of 1.40-1.50 consistently obtained with the stock diet (Tables 37, 38 and 39), while the high-protein diet has increased it to 1.55 to 1.72 (Tables 30 and 32). Clearly, therefore, the RNA content per cell in the kidney of animals on the stock diet can be elevated by increasing their protein intake or depressed by diminishing it.

3.2. The effect of starvation.

Since the feeding of a protein-free diet slightly depresses the RNA content per cell, it is possible that starvation will considerably magnify this effect, for the animal is being deprived not only of dietary protein but also of all the other nutrients essential for growth. As has been reported in the Introduction (Section 1.4.1.), some workers have reported that starvation completely inhibits compensatory renal hypertrophy if the growth of the remaining kidney is followed by measuring changes in mitotic activity. It seemed, therefore, of considerable interest in the present investigation to find how starvation might affect the chemical changes associated with compensatory renal hypertrophy.

Accordingly 36 male rats weighing 140 to 150 g. were subjected to right unilateral nephrectomy. They were then divided randomly into two groups. Post-operatively, one of the groups was given food ad libitum, the food intake of each individual animal being measured: the other group was starved. After 12, 24 and 36 hours, six animals from each of the groups were killed and their remaining kidneys analysed. Six unoperated animals were also sacrificed as a zero time control. The results are given in Tables 33 to 36 inclusive. It should be pointed out that the kidneys of the animals within each time interval were analysed together, and can therefore be compared directly with one another. Comparison of the absolute results of one time interval with those of another, is, however, of doubtful significance, because of possible variation between one batch of analysis and another. The difference in size and composition between the right and left kidneys at any one time interval can, however, be compared with the difference at another. Accordingly these differences were calculated as percentages and plotted against the time after unilateral nephrectomy. These results are shown in Figures 21, 22 and 23.

The fed animals, sacrificed 12 hours post-operatively, ate 1.8 ± 0.21 g. of food between operation and death. The animals sacrificed after 24 hours ate 6.4 ± 0.81 g. while those sacrificed after 36 hours ate 11.7 ± 1.04 g. in the same period. Both fed and starved groups lost weight post-operatively, the starved animals to

Table 33

Starvation experiment. The weight and composition of right and left kidneys of male rats.

	Right Kidney	Left Kidney
Weight (mg.)	606 ± 14.8	566 ± 13.9
DNAP		
μg/100 mg. kidney	27.4 ± 0.53	27.8 ± 0.65
μg/kidney	166 ± 4.4	157 ± 3.8
RNAP		
μg/kidney	239 ± 7.4	226 ± 5.4
μg/μg. DNAP	1.44 ± 0.030	1.44 ± 0.035
Protein		
mg/kidney	87 ± 2.3	81 ± 1.1
μg/μg. DNAP	525 ± 4.9	513 ± 8.2

Values are means ± S.E.M. for six animals weighing between 134 and 155 g.

Table 34

Starvation experiment. The effect of starvation and right unilateral nephrectomy on the size and composition of the left kidney after 12 hours.

	Fed		Starved	
	Right Kidney	Left Kidney	Right Kidney	Left Kidney
Weight (mg.)	584 ± 26.9	576 ± 28.2	599 ± 18.2	586 ± 18.4
DNAP				
μg/100 mg. kidney	22.3 ± 0.72	21.8 ± 1.00	22.7 ± 0.57	22.2 ± 0.65
μg/kidney	129 ± 5.5	124 ± 3.3	136 ± 2.8	130 ± 0.9
RNAP				
μg/kidney	169 ± 5.3	168 ± 4.0	176 ± 4.4	175 ± 3.9
μg/μg. DNAP	1.32 ± 0.019	1.37 ± 0.033	1.30 ± 0.026	1.35 ± 0.032
Protein				
mg/kidney	91 ± 4.4	87 ± 3.8	93 ± 2.3	90 ± 1.5
μg/μg. DNAP	711 ± 27.7	707 ± 28.1	686 ± 16.8	692 ± 9.3

Values are means ± S.E.M. for six animals weighing between 140 and 174 g.

Table 35

Starvation experiment. The effect of starvation and right unilateral nephrectomy on the size and composition of the left kidney after 24 hours.

	Fed		Starved	
	Right Kidney	Left Kidney	Right Kidney	Left Kidney
Weight (mg.)	608 ± 29.5	622 ± 25.3	639 ± 23.7	626 ± 19.5
DNAP				
μg/100 mg. kidney	25.1 ± 0.60	22.9 ± 0.69	25.4 ± 0.81	24.2 ± 0.67
μg/kidney	153 ± 10.0	142 ± 7.7	162 ± 5.3	152 ± 6.4
RNAP				
μg/kidney	231 ± 5.0	259 ± 17.0	236 ± 11.0	256 ± 15.1
μg/μg. DNAP	1.51 ± 0.060	1.82 ± 0.090	1.46 ± 0.046	1.69 ± 0.044
Protein				
mg/kidney	97 ± 4.1	99 ± 3.2	102 ± 3.2	99 ± 3.8
μg/μg. DNAP	644 ± 22.0	704 ± 22.0	640 ± 17.1	655 ± 13.8

Values are means ± S.E.M. for six animals weighing between 141 and 168 g.

Table 36

Starvation experiment. The effect of starvation and right unilateral nephrectomy on the size and composition of the left kidney after 36 hours.

	Fed		Starved	
	Right Kidney	Left Kidney	Right Kidney	Left Kidney
Weight (mg.)	575 ± 16.6	633 ± 25.7	605 ± 24.9	636 ± 19.5
DNAP				
μg/100 mg. kidney	25.8 ± 1.06	22.0 ± 1.04	25.5 ± 1.05	23.4 ± 0.95
μg/kidney	149 ± 7.3	139 ± 8.2	155 ± 8.7	149 ± 7.0
RNAP				
μg/kidney	204 ± 11.7	254 ± 19.0	209 ± 10.3	246 ± 10.6
μg/μg. DNAP	1.37 ± 0.019	1.82 ± 0.068	1.37 ± 0.037	1.66 ± 0.046
Protein				
mg/kidney	95 ± 2.4	101 ± 1.9	100 ± 3.3	98 ± 4.1
μg/μg. DNAP	650 ± 42.0	741 ± 35.6	657 ± 31.6	664 ± 28.4

Values are means ± S.E.M. for six animals weighing between 129 and 157 g.

a greater extent than the fed animals (Figure 21). Even in the first 12 hours post-operatively, both groups significantly lost weight ($P < 0.001$) though at this time there was no significant difference between the groups. This lack of difference was scarcely surprising since the fed animals had only eaten about 2 g. of food by this time. By 24 hours after the operation, the loss of body weight in the fed group had more or less ceased but the starved animals continued to lose weight. This difference was magnified at 36 hours.

Figure 21 shows that despite the loss in body weight, the kidney weight and mean cell mass of the remaining kidney increased in both groups. The increase was significant by 12 hours and continued up to 36 hours, reaching values about 20% above normal in the fed animals and about 10% above normal in the starved animals. Thus there appeared to be a difference in the response of the two groups, but this was not significant. Figure 22 shows that there was no significant change in the total kidney content of DNA at any time in either group. Since changes in DNA have not been detected in previous experiments (Table 21) before at least 48 hours post-operatively, this result is hardly surprising. Figure 22 also shows that changes in the total content of RNA, of the order expected from past results (Section 3.2.3.), were obtained. There was no significant difference in this response between the two groups. The changes in protein content of the remaining kidneys, however, presented a different pattern. The fed animals showed a 9% increase

Figure 21

The effect of starvation on rat body weight and on kidney weight and mean cell mass after right unilateral nephrectomy.

The results are expressed as the percentage difference between the surviving kidney removed at death and the kidney excised at operation.

●——● mean cell mass, rats fed post-operatively.

○– – –○ mean cell mass, rats starved post-operatively.

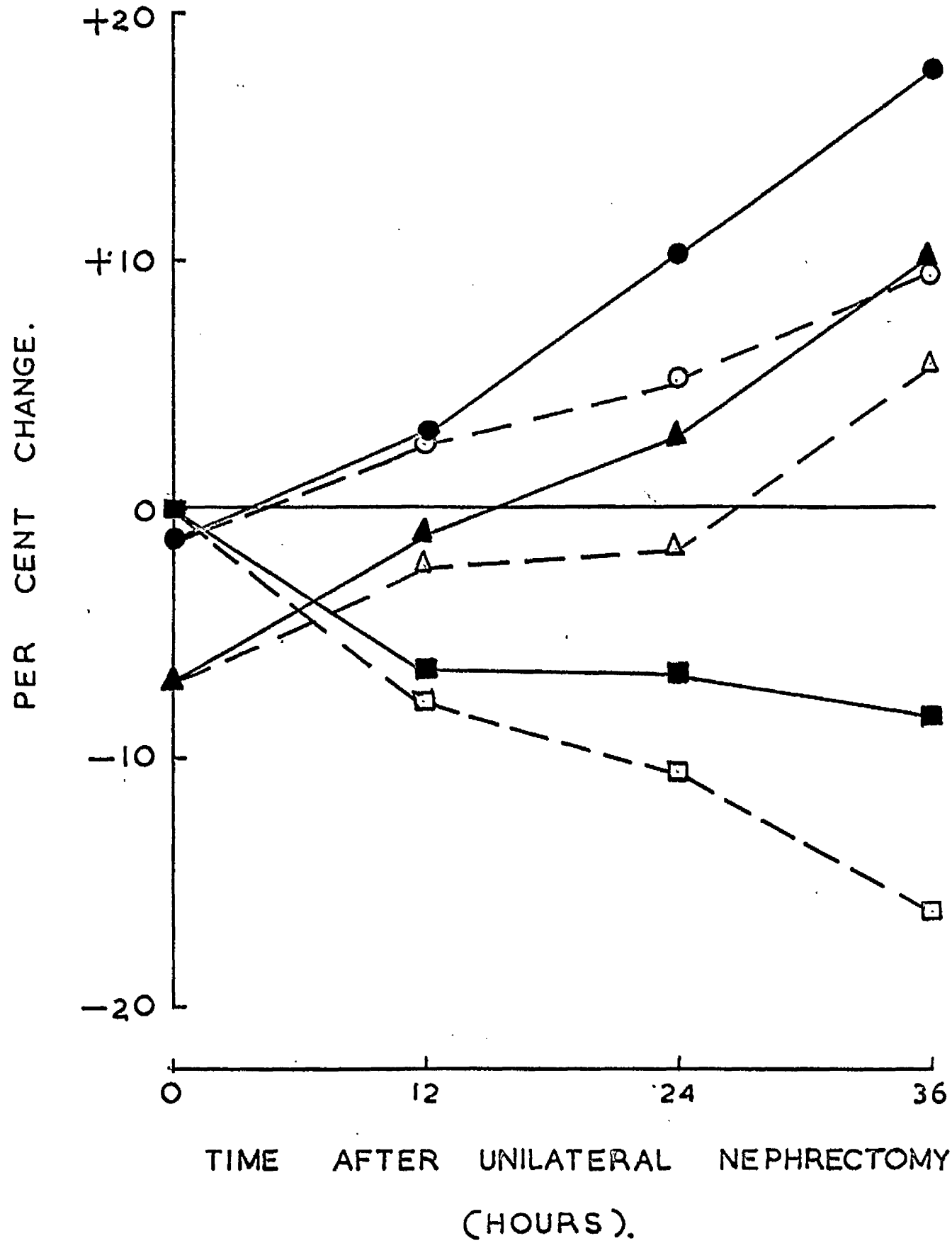
▲——▲ kidney weight, rats fed post-operatively.

△– – –△ kidney weight, rats starved post-operatively.

■——■ body weight of fed rats.

□– – –□ body weight of starved rats.

FIGURE 21.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+10
0
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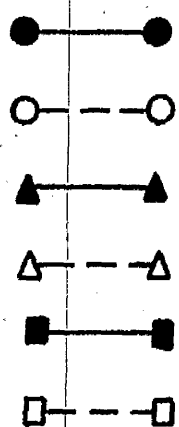
PER CENT CHANGE.
0
12
24
36
TIME AFTER UNILATERAL NEPHRECTOMY
(HOURS).

Figure 22

The effect of starvation on the composition of the remaining kidney after right unilateral nephrectomy.

The results are expressed as the percentage difference between the surviving kidney removed at death and the kidney excised at operation.



RNAP/kidney, rats fed post-operatively.

RNAP/kidney, rats starved post-operatively.

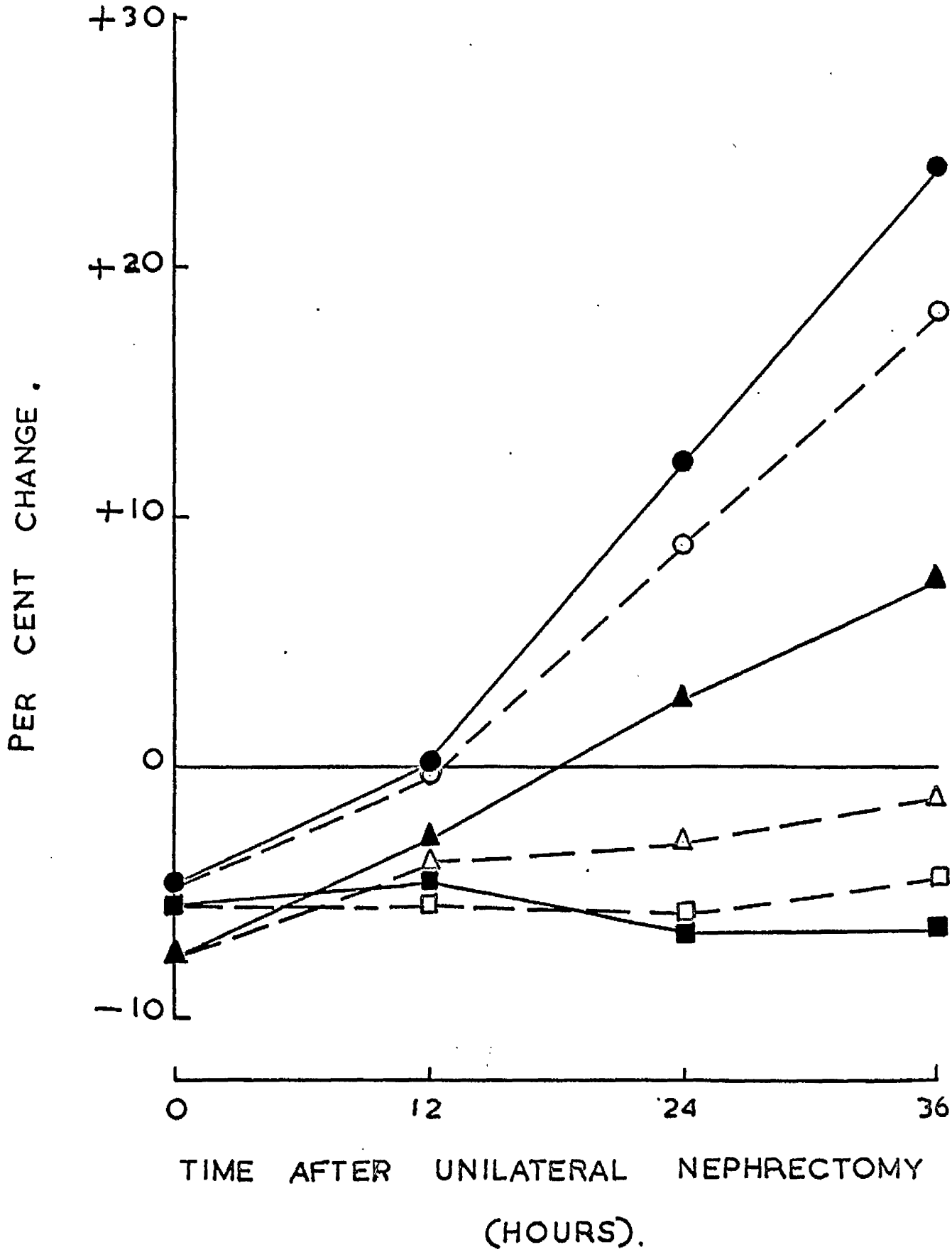
protein/kidney, rats fed post-operatively.

protein/kidney, rats starved post-operatively.

DNAP/kidney, rats fed post-operatively.

DNAP/kidney, rats starved post-operatively.

FIGURE 22.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PER CENT CHANGE.
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12
24
36
TIME AFTER UNILATERAL NEPHRECTOMY
(HOURS).

in protein content at 24 hours ($P < 0.05$) and a 13% increase at 36 hours ($P < 0.01$) after the operation. The starved animals, however, showed no change in protein content at 24 hours and only a 5% increase at 36 hours ($P < 0.05$). If one looks at the mean cell composition (Figure 23) one finds that both groups showed a steady increase in RNA/DNA ratio after the operation which was not significant at 12 hours but was significant at 24 hours ($P < 0.001$) and 36 hours ($P < 0.001$). Although the changes in the fed group appeared to be larger than in the starved group, the differences between the groups were not significant. The changes in protein content per cell gave quite a different picture. The fed group showed a steady change in protein content per cell, the increase being significant at 24 hours ($P < 0.05$) and at 36 hours ($P < 0.001$). The starved group, however, showed no significant changes.

Thus the results of this experiment can be summed up by saying that during the first 36 hours after unilateral nephrectomy, starvation does not significantly affect the increase in cell mass or in content of RNA of the surviving kidney, but it does apparently abolish the increase in protein content per cell. This is a little surprising. It has been known for many years that starvation does diminish the protein content of some organs, notably the liver (Addis, Poo and Lew, 1936a, b; Thomson et al., 1953; Munro, 1964a) but generally speaking, the fall in protein per cell is accompanied by an equivalent fall in RNA per cell (Allison, Wannemacher and

Figure 23

The effect of starvation on the RNA and protein contents per cell of the remaining kidney after right unilateral nephrectomy.

The results are expressed as the percentage difference between the surviving kidney removed at death and the kidney excised at operation.

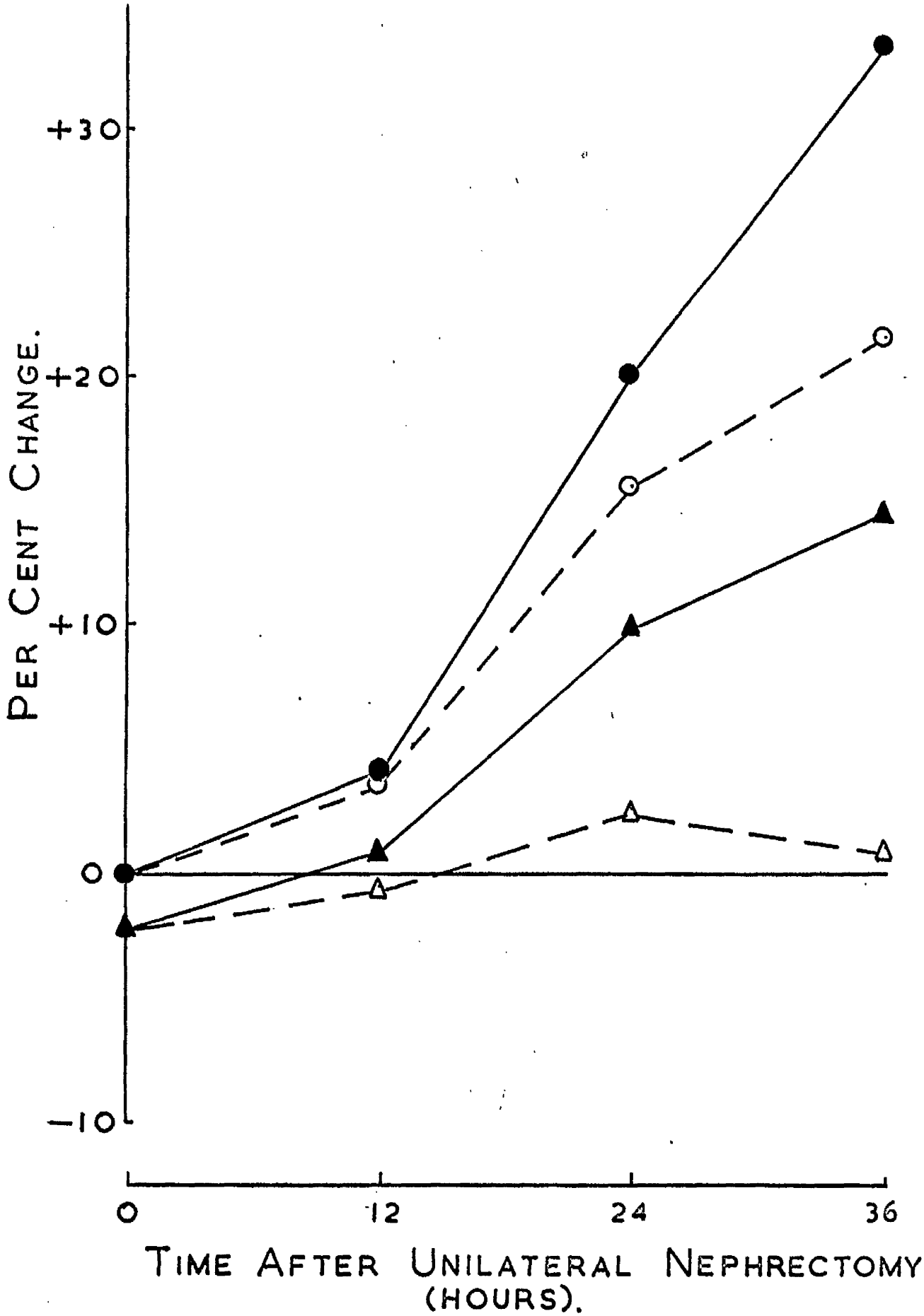
●——● RNAP/DNAP ratio, rats fed post-operatively.

○— —○ RNAP/DNAP ratio, rats starved post-operatively.

▲——▲ protein/DNAP ratio, rats fed post-operatively.

Δ— —Δ protein/DNAP ratio, rats starved post-operatively.

FIGURE 23.


+30
+20
+10
0
−10
PER CENT CHANGE.
0
12
24
36
TIME AFTER UNILATERAL NEPHRECTOMY
(HOURS).

Banks, 1963). In the present instance, however, it is important to remember that the animals were starved for only a very short period and that if starvation had been prolonged, additional changes in composition, including an effect on RNA, might have become apparent. However that may be, the important practical conclusion that comes out of this experiment, is that the increase in RNA content per cell, which is the most dramatic early change in compensatory renal hypertrophy, is not significantly affected by starvation. To that extent, the RNA/DNA ratio is a more reliable indicator of compensatory renal hypertrophy than is mitotic index.

3.3. The effect of a urea-containing diet.

It is apparent from the preceeding sections that increasing the protein content of the diet produces some of the changes in kidney size and composition associated with compensatory renal hypertrophy. On the other hand, it is quite clear that the early changes at least, are not inhibited by starvation, or even significantly depressed by it. It does look, therefore, as though the early stages of compensatory renal hypertrophy proceed without much regard to the nutritional status of the animal. There remains the question of whether the changes in the kidneys of intact rats produced by diets high in protein, are due to the extra urea which has to be excreted. If this is the case, then the addition of a substantial amount of urea to the diet of normal animals should also lead to kidney hypertrophy. The effect of supplementing the diet with an amount

of urea roughly equivalent to three times the protein it already contained was therefore investigated. The animals receiving this diet presumably had to excrete four times the normal daily amount of urea. Figure 24 shows that this was indeed the case. The diet also increased urine volume roughly in proportion.

The effect of the diet, however, on kidney size and composition, over a four day period, was relatively small (Table 37). No significant difference was found in kidney weight or in the total content of DNA, but the DNA concentration decreased ($P < 0.02$). In addition, there were significant increases in the RNA/DNA ($P < 0.002$) and protein/DNA ($P < 0.05$) ratios. Although these changes are similar to the effects produced in the remaining kidney after unilateral nephrectomy (Table 38), they are only about one third as great. It seems unlikely, therefore, that the hypertrophy of the remaining kidney after unilateral nephrectomy is due simply to the increased amount of urea it has to excrete.

3.4. The effect of dietary salt.

After unilateral nephrectomy the remaining kidney has to take on an additional load in respect of all its functions. It is possible, therefore, that some function other than the excretion of urea may be the controlling factor in compensatory renal hypertrophy. Two possibilities are electrolyte excretion and the maintainance of acid-base balance. Accordingly these were investigated by determining the effect, on normal unoperated animals, of supplementing the diet with

Figure 24

The effect of a urea - containing diet on urine volume and on urinary urea output per day.

Each point represents the mean value for six animals weighing between 146 and 152 g. The animals were fed 12 g. of the stock diet (Diet 41) per day for 4 days. Between the fourth and ninth days (marked by arrows) they were fed a diet consisting of 90% stock diet and 10% by weight urea. The line marked XY represents the average urea output on the stock diet. The line marked AB represents the estimated urea output on the urea diet, assuming that all the urea ingested was excreted.

●——● represents urea excretion per day.

O— —O represents urine volume per day.

FIGURE 24.

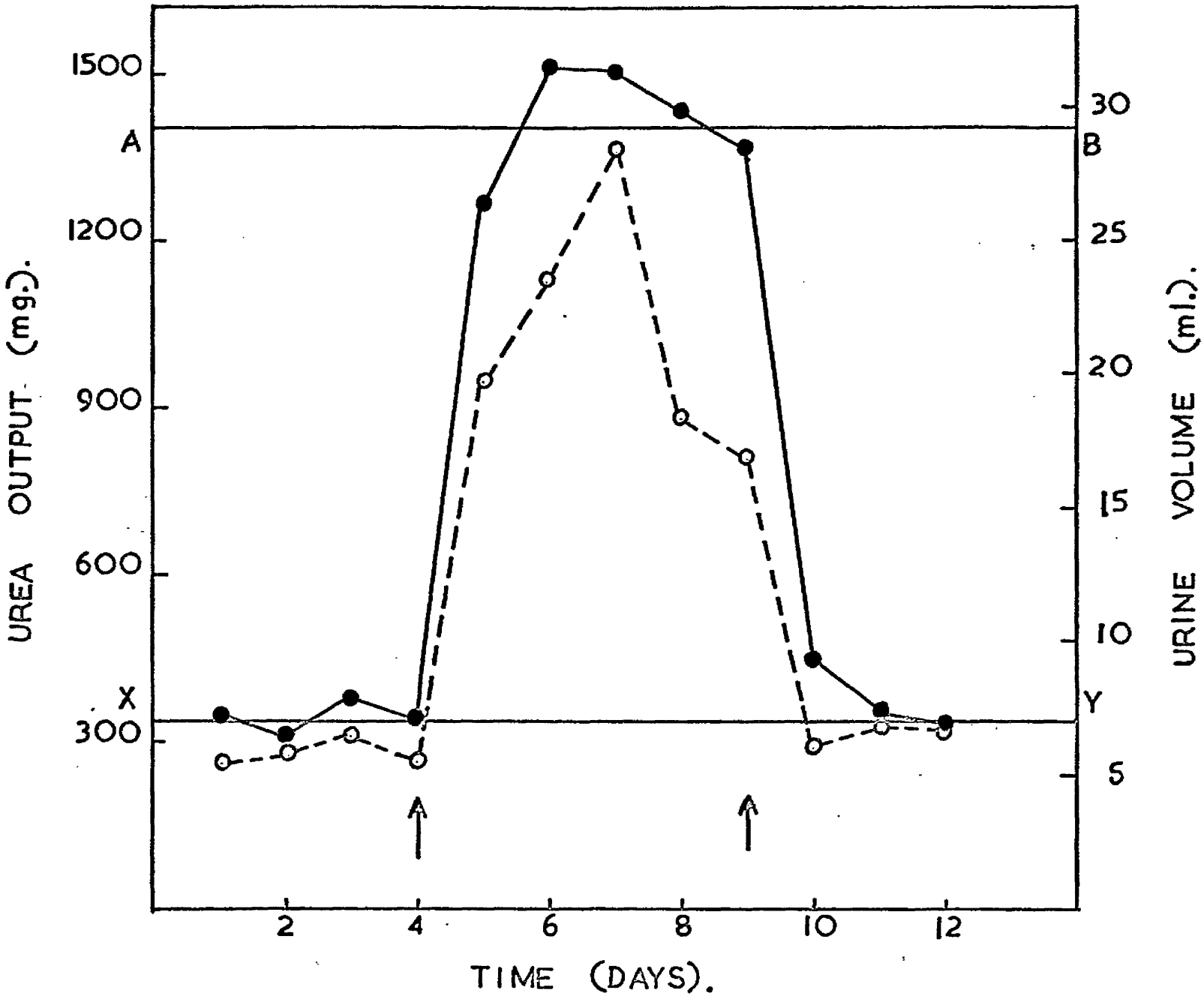

1500
A
1200
900
600
X
300
UREA OUTPUT (mg.).
30
B
25
20
15
10
Y
5
URINE VOLUME (ml.).
2
4
6
8
10
12
TIME (DAYS).

Table 37

The effect of a urea-containing diet on the size and composition of the left kidney.

	Diet	
	Stock Diet	Stock Diet + 10 per cent urea
Body Weight (g.)	157 ± 8.0	157 ± 8.3
Kidney Weight (mg.)	579 ± 27.2	627 ± 16.8
DNAP		
µg/100 mg. kidney	33.4 ± 0.46	31.0 ± 0.67†
µg/kidney	194 ± 7.1	194 ± 5.3
RNAP		
µg/kidney	274 ± 12.8	300 ± 7.6
µg/µg. DNAP	1.40 ± 0.02	1.55 ± 0.03*
Protein		
mg/kidney	101 ± 3.6	108 ± 1.8
µg/µg. DNAP	520 ± 7.2	557 ± 10.5‡

Values are means ± S.E.M. for six animals weighing between 131 and 188 g. For the purpose of statistical comparison, the animals were paired according to body weight.

The urea diet consisted of the stock diet 41 B (Protein content 13.7%) containing 10 per cent by weight urea. The feeding of this diet gave a nitrogen intake of 3,400 mg. over the four days of the experiment; the nitrogen intake of the basal diet was 1,100 mg. over the same period.

* Significantly different from the value for the group fed diet 41 B with a P value of 0.002 or less.

† Ditto, with a P value of 0.02 or less.

‡ Ditto, with a P value of 0.05 or less.

Table 38

The effect of right unilateral nephrectomy on the size and composition of the left kidney after 96 hours.

	Right Kidney	Left Kidney
Weight (mg.)	612 ± 35.4	799 ± 35.3*
DNAP		
µg/100 mg. kidney	33.7 ± 0.46	30.2 ± 0.52‡
µg/kidney	207 ± 12.4	242 ± 13.4†
RNAP		
µg/kidney	299 ± 17.7	472 ± 23.6*
µg/µg. DNAP	1.45 ± 0.03	1.96 ± 0.01*
Protein		
mg/kidney	103 ± 5.9	128 ± 6.4*
µg/µg. DNAP	502 ± 16.4	534 ± 10.9†

Values are means ± S.E.M. for five male rats weighing between 136 and 171 g.

* Significantly different from the value for the right kidney with a P value of 0.001 or less.

† Ditto, with a P value of 0.002 or less.

‡ Ditto, with a P value of 0.01 or less.

various salts.

Electrolyte balance is largely a matter of sodium excretion or retention. In addition, sodium ions are the main cations of the urine. For these reasons, one of the diets used contained sodium chloride which would increase the amount of sodium which the kidney would have to excrete and this in turn would increase the water output. Ammonium chloride was added to stock diet and fed to another group of animals, since this is a well known technique for producing an acidosis. The acidosis arises from the fact that in the conversion of the ammonium ion to urea, a hydrogen ion is released. A third group was fed a diet containing ammonium citrate as a control for the ammonium chloride fed group. This salt is completely metabolised in vivo. The ammonium ions are converted to urea, which is excreted in the urine, with the release of a hydrogen ion, as with the ammonium chloride diet. No acidosis results, however, for the citrate ion is metabolised via the citric acid cycle, giving rise to carbon dioxide and water. This in turn would provide a bicarbonate anion which would neutralise the hydrogen ion produced.

The concentrations of these salts in the diets used, was dictated by the amounts which the animals would tolerate. Sodium chloride and ammonium citrate were fairly well tolerated. Diets containing substantial amounts of ammonium chloride were not; diets containing 3% by weight were the maximum the animals would accept. Accordingly this was used. The ammonium citrate diet used (7% by

weight) gave a nitrogen intake equivalent to that of the 3% ammonium chloride diet. The sodium chloride diet used (3.3% by weight) was equimolar to the 3% ammonium chloride diet. These three diets were fed to groups of animals for a period of six days and the size and composition of the left kidneys was then compared with the size and composition of the kidneys of a fourth group fed a stock diet.

Figure 25 shows that the sodium chloride and ammonium chloride diets increased urine volume by 2 to 3-fold. The feeding of these diets therefore, should give an indication of any effect of increased urine excretion. In addition, the ammonium chloride did produce an acidosis, reflected in an 8-fold increase in urinary ammonia (Figure 26). Because of possible bacterial contamination, these results may not be too reliable, but there can be no doubt that the ammonium chloride diet does produce an acidosis. Figure 27 shows that the ammonium chloride and ammonium citrate diets, which would increase the nitrogen intake of the animals, increased the urea output. The increased output was roughly in proportion to the intake.

The effect of these diets on kidney composition is shown in Table 39. The sodium chloride diet had no apparent effect on kidney size or composition. Thus the amount of chloride ion to be excreted has no effect on the size of the kidney, nor indeed, bearing in mind the results of the urea diet which also produced a slight diuresis (Figure 24), has the amount of water to be excreted. The ammonium chloride diet on the other hand, did have a marked effect on the

Figure 25

The effect of high salt diets on urine volume per day.

Each point represents the mean for 3 animals weighing between 165 and 180 g. The first group of animals were fed the stock diet (Diet 41) for the period of the experiment. All other animals were fed the stock diet for the first 4 days. Between the fourth and eighth days (marked by arrows) they were fed the salt diets as indicated. The sodium chloride diet contained 96.7% stock diet and 3.3% sodium chloride. The ammonium chloride diet contained 97% stock diet and 3% ammonium chloride. The ammonium citrate diet contained 93% stock diet and 7% ammonium citrate.

FIGURE 25.

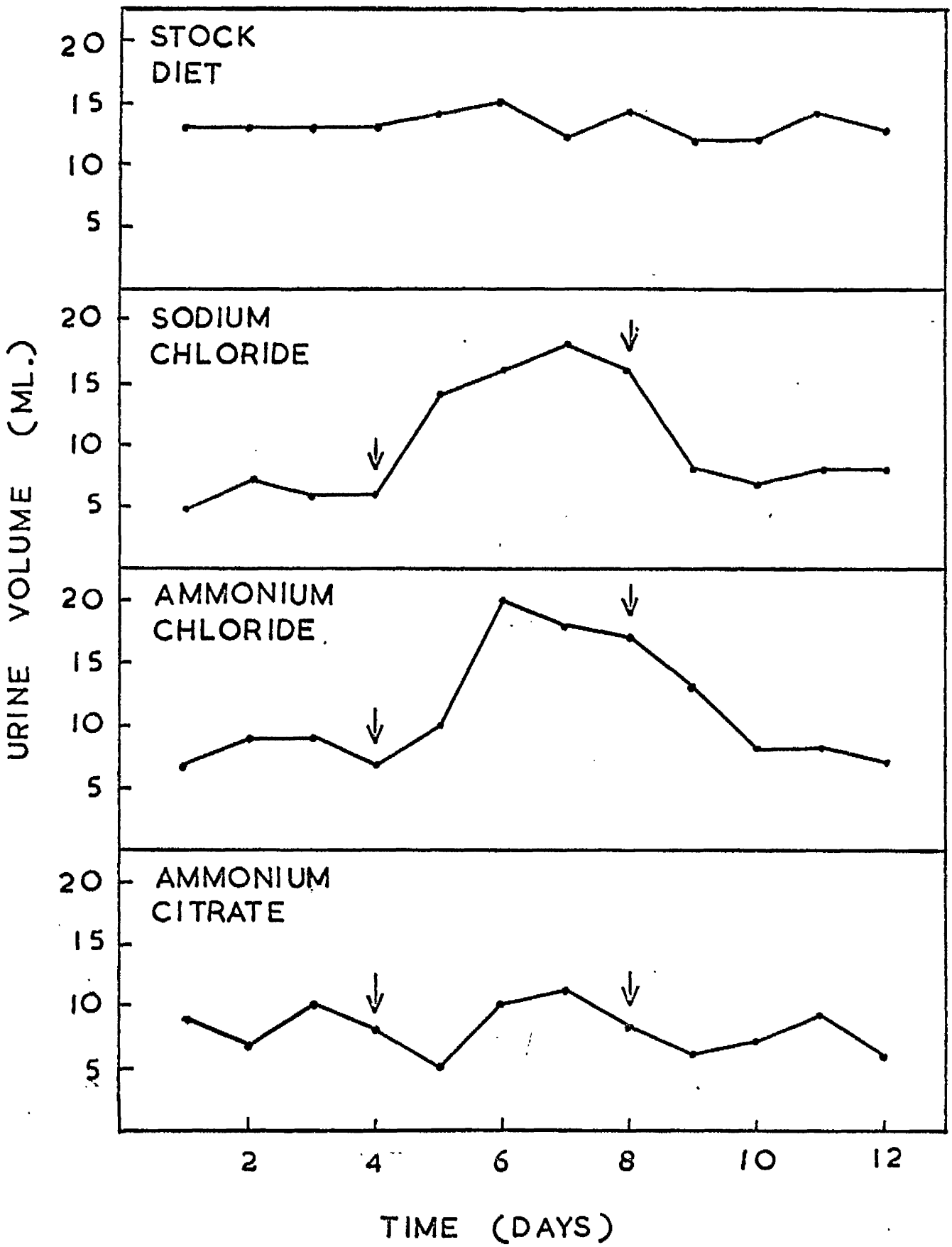

STOCK DIET
SODIUM CHLORIDE
AMMONIUM CHLORIDE
AMMONIUM CITRATE
20
15
10
5
2
4
6
8
10
12
URINE VOLUME (ML.)
TIME (DAYS)

Figure 26

The effect of high salt diets on the daily urinary ammonia output.

Each point represents the mean for 3 animals weighing between 165 and 180 g. The diets used were as described in Figure 25.

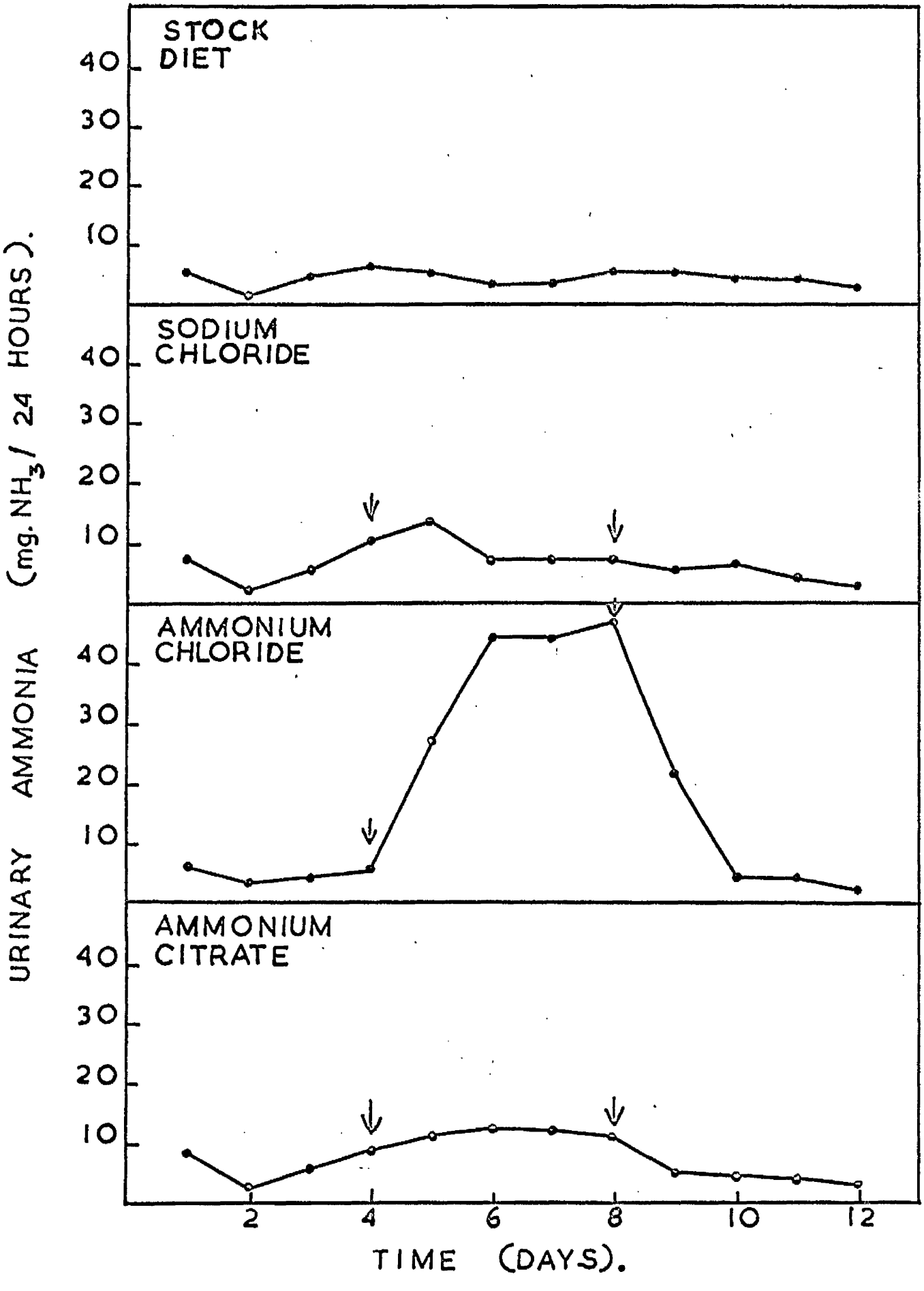

STOCK DIET
SODIUM CHLORIDE
AMMONIUM CHLORIDE
AMMONIUM CITRATE
40
30
20
10
2
4
6
8
10
12
URINARY AMMONIA (mg. NH_3 / 24 HOURS).
TIME (DAYS).

Figure 27

The effect of high salt diets on the daily urinary urea output.

Each point represents the mean for 3 animals weighing between 165 and 180 g. The diets used were as described in Figure 25. The nitrogen intake of the stock diet was 260 mg./day; that of the ammonium chloride diet was 349 mg./day and that of the ammonium citrate diet was 348 mg./day.

FIGURE 27.

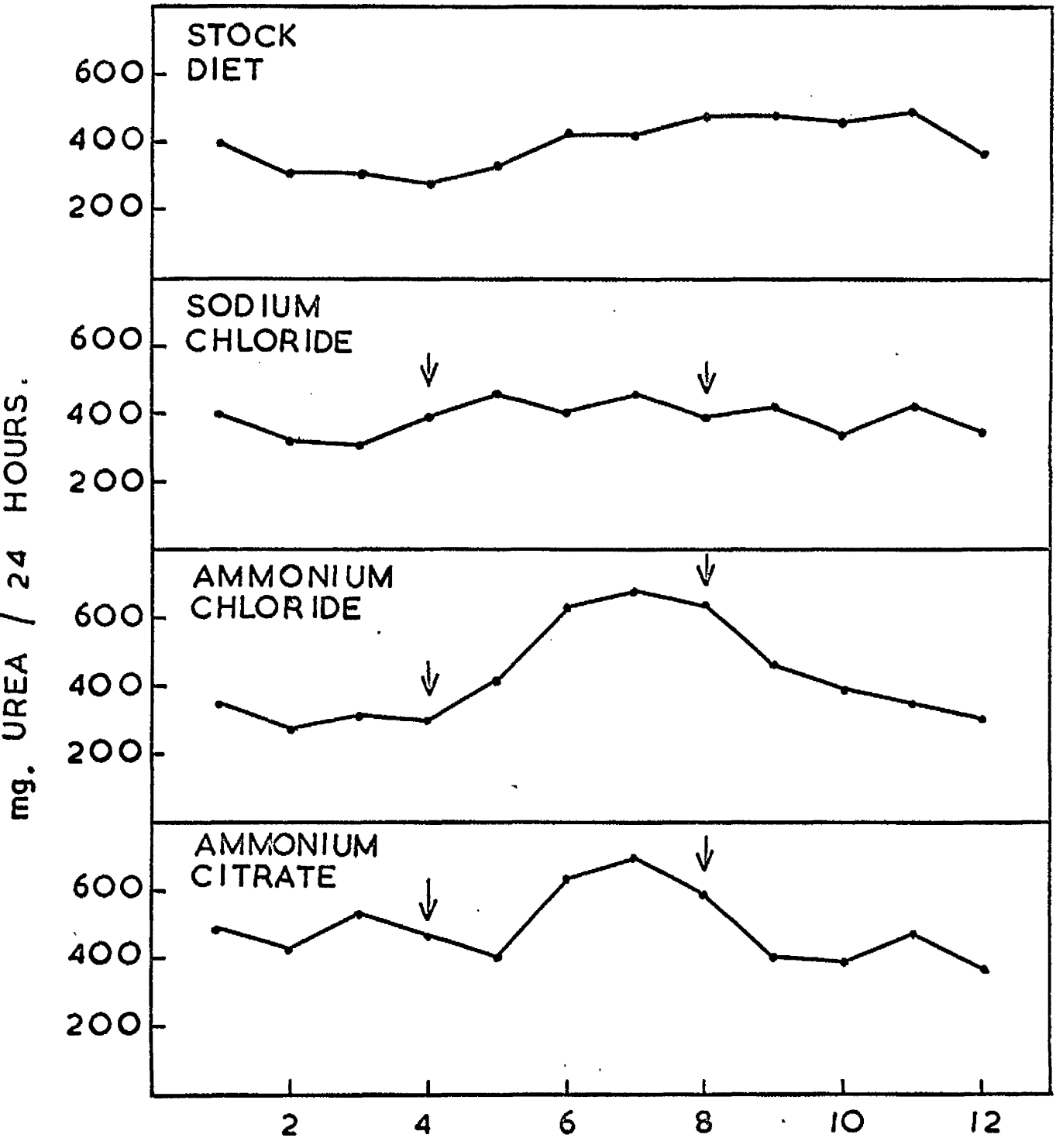

STOCK DIET
SODIUM CHLORIDE
AMMONIUM CHLORIDE
AMMONIUM CITRATE
600
400
200
mg. UREA / 24 HOURS.
2
4
6
8
10
12
TIME (DAYS).

Table 39

The effect of high salt diets on the weight and composition of the left kidney after 6 days.

Diet	Left Kidney Weight (mg.)	DNAP		RNAP		Protein	
		ug/100 mg. Wet Weight	ug/kidney	ug/kidney	ug/ug. DNAP	mg/kidney	ug/ug. DNAP
Diet 41B	663 ± 27.0	32.2 ± 1.4	213 ± 12.9	317 ± 10.3	1.50 ± 0.07	123 ± 6.5	580 ± 20.4
3.3% Sodium Chloride	708 ± 24.4	33.4 ± 1.1	237 ± 14.8	341 ± 10.8	1.46 ± 0.05	128 ± 4.5	546 ± 21.9
3% Ammonium Chloride	835 ± 21.8*	27.2 ± 1.1§	226 ± 8.2	398 ± 12.1*	1.77 ± 0.05†	155 ± 5.4†	690 ± 31.3§
7% Ammonium Citrate	744 ± 15.6§	30.4 ± 1.2	225 ± 7.3	352 ± 8.6§	1.58 ± 0.07	131 ± 2.9	587 ± 23.0

Values are means ± S.E.M. for six animals weighing 175 to 215 g. They were offered 12 g. of diet per day. The first group therefore received 12 g of stock diet (diet 41B) per day; the second group received 11.60 g. stock diet and 0.40 g. sodium chloride; the third group received 11.64 g. stock diet and 0.36 g. ammonium chloride and the fourth group received 11.16 g. stock diet and 0.84 g. ammonium citrate.

* Significantly different from the value of the animals on the stock diet with a P value of 0.001 or less.

† Ditto, with a P value of 0.01 or less.

‡ Ditto, with a P value of 0.02 or less.

§ Ditto, with a P value of 0.05 or less.

kidney, producing a significant increase in kidney weight ($P < 0.001$). This was not due to an increase in cell number, for the total content of DNA did not alter, but the DNA concentration decreased ($P < 0.05$), indicating that the mean cell mass had increased. This was reflected in an increase in the total content of RNA ($P < 0.001$) and protein ($P < 0.01$) and in significantly larger RNA/DNA ($P < 0.02$) and protein/DNA ratios ($P < 0.05$). These changes were not due to the ammonium ion, for the ammonium citrate diet produced only one significant change, namely a small increase in the total content of RNA ($P < 0.05$). Thus the kidney hypertrophy produced by the ammonium chloride diet cannot be due to increased electrolyte excretion or to increased urea excretion or to increased water excretion, and must therefore be a result of the acidotic effect of the ammonium chloride.

3.5. Conclusions.

The experiments in this section have shown that increases in kidney cell size and composition can be brought about not only by unilateral nephrectomy but also in response to variations in the diet. It is obviously important to decide how far the effects of diet are identical with those of unilateral nephrectomy. For ease of comparison, the relevant information has been collected from the appropriate Tables and set out in a summary in Table 40.

Two important generalisations can be drawn from Table 40. The first of these is that broadly speaking, changes in mean cell mass are in all cases paralleled by a roughly equivalent change in the

Table 40

Comparison of changes in kidney cell size and composition following different treatments.

Treatment	Time	Changes in Mean Cell Mass(%)	Changes in Protein Per Cell(%)	Changes in RNA Per Cell(%)	Original Table Number
Unilateral Nephrectomy	12 Hours	1	1	7	23
Ditto	24 Hours	16	-	25	22
Ditto	2 Days	0	-	32	18
Ditto	2 Days	19	24	39	20
Ditto	2 Days	14	8	22	41
Ditto	2 Days	9	12	23	49
Ditto	4 Days	6	5	29	19
Ditto	4 Days	11	6	35	38
Ditto*	4 Days*	10*	5*	16*	30*
Ditto†	4 Days†	21†	24†	26†	30†
Protein Content of Diet Varied	4 Days	21	21	24	32
Protein Content of Diet Varied	8 Days	24	29	26	32
Urea Added To Diet	4 Days	8	7	11	37
Ammonium Chloride Added to Diet	6 Days	18	19	18	39

All unilaterally nephrectomized animals were on a stock diet except those marked thus *, which were on a protein-free diet and those marked thus †, which were on a high-protein diet.

protein content per cell. In other words, changes in kidney weight are, in general, due to changes in the protein content rather than to changes in, for example, water or fat. The second main point is that if the mean cell mass of the kidneys is increased by modifying the diet, the RNA content per cell and the protein content per cell are affected to approximately the same extent. Thus 4 days on a high-protein diet results in about 21 to 24% more RNA and protein than on a protein-free diet; 8 days on the high-protein diet results in 26 to 29% more RNA and protein than 8 days on the protein-free diet; 6 days on an ammonium chloride diet results in an increase of 18 to 19% in RNA and protein. The results with a urea diet may or may not fit into this general picture, the changes obtained were too small to draw any firm conclusions. On the other hand, after unilateral nephrectomy, the increase in RNA per cell is normally very much greater than the increase in protein per cell. The only exception to this generalisation is that if an animal is unilaterally nephrectomised and maintained on a high-protein diet, the increase in RNA per cell is the same as the increase in protein per cell. This distinction between the changes produced by nephrectomy and those produced by dietary means, together with the fact that although the total DNA content of the remaining kidney has been shown to increase after unilateral nephrectomy, similar changes were never obtained following modifications of the diet, gives additional evidence for the view that compensatory renal hypertrophy cannot be

explained satisfactorily on the grounds that the remaining kidney has to excrete more salt, or more water, or more acid, or more urea.

4. The effect of renal decapsulation on compensatory renal hypertrophy.

The kidneys of normal animals are each covered by a closely fitting capsule. This is composed mainly of fibrous connective tissue in which the collagen fibres greatly outnumber the elastic fibres (Garven, 1957). It is, therefore, relatively inelastic. It is possible that the capsule might exert some control on the magnitude of compensatory renal hypertrophy by compressing the expanding kidney. This idea prompted Allen and Mann (1955) to investigate, in rabbits, the effect of renal decapsulation on the size of the hypertrophic response to unilateral nephrectomy. They unilaterally nephrectomized a pair of animals on the right and at the same time decapsulated the left kidneys. After 6 months the animals were killed. The weight of the decapsulated kidney was, on average, about 40% greater than the weight of the surviving kidney in control animals which had been unilaterally nephrectomized without decapsulation of the left kidney. It seemed possible, therefore, that the capsule might have at least some effect on the size of the kidney remaining after unilateral nephrectomy and it appeared worthwhile to investigate the effect of renal decapsulation on compensatory renal hypertrophy at shorter time intervals.

Twelve male rats weighing 180 to 220 g. were divided into 4

groups. In the first group, a sham operation simulating a right unilateral nephrectomy was carried out. The second group was subjected to right unilateral nephrectomy. The third group were sham operated on the right and at the same time, the capsule was removed from the left kidney. In the fourth group, right unilateral nephrectomy was performed together with the removal of the capsule from the left kidney. After 48 hours the animals were killed and their kidneys analysed. The results are shown in Table 41. The size and composition of the kidneys of the sham operated group were in good agreement with the figures obtained from normal rats (Table 13). The sham operation, therefore, had no effect on the kidneys. The remaining kidneys of the unilaterally nephrectomized animals responded as one would have expected from past results (Table 21), with a large increase in kidney weight and RNA content per kidney and per cell. Although there was only a small increase in DNA content, the DNA concentration showed a marked decrease, consistent with the idea that the mean cell mass had increased in preparation for DNA synthesis and cell division. There was in addition, a small increase in the protein content of the remaining kidney. Table 41 also shows that decapsulation of the left kidney at the time of operation, clearly had little or no effect on the size or composition of the kidneys of sham operated animals or on the magnitude of compensatory renal hypertrophy in the unilaterally nephrectomized animals. It would seem most unlikely, therefore, that the kidney

Table 41

The effect of decapsulation of the left kidney on compensatory renal hypertrophy 48 hours after right unilateral nephrectomy. Values are the means for 3 animals.

Treatment	Rat Body Weight (g.)	Kidney	Kidney Weight (mg.)	DNAP		RNAP		Protein	
				µg/100 mg. Kidney	µg/ kidney	µg/ kidney	µg/µg. DNAP	mg/ kidney	µg/µg. DNAP
Right Sham Operation	214	Right	739	28.8	212	358	1.69	130	613
		Left	679	29.6	201	332	1.66	125	623
		Ratio $\frac{\text{Left}}{\text{Right}}$	0.92	1.03	0.95	0.93	0.98	0.96	1.02
Right Unilateral Nephrectomy	209	Right	638	29.9	192	301	1.59	124	652
		Left	733	25.8	190	366	1.92	132	701
		Ratio $\frac{\text{Left}}{\text{Right}}$	1.15	0.86	0.99	1.22	1.21	1.06	1.08
Right Sham Operation, Left Decapsulation	213	Right	751	28.5	214	357	1.67	131	611
		Left	716	29.8	212	359	1.69	134	630
		Ratio $\frac{\text{Left}}{\text{Right}}$	0.95	1.05	0.99	1.01	1.01	1.02	1.03
Right Unilateral Nephrectomy, Left Decapsulation	218	Right	673	28.8	193	322	1.66	119	606
		Left	780	26.7	209	405	1.95	140	671
		Ratio $\frac{\text{Left}}{\text{Right}}$	1.16	0.93	1.08	1.26	1.17	1.18	1.11

capsule has any effect on the degree of compensatory renal hypertrophy, at least in the short term after the operation.

5. Early chemical changes in compensatory renal hypertrophy.

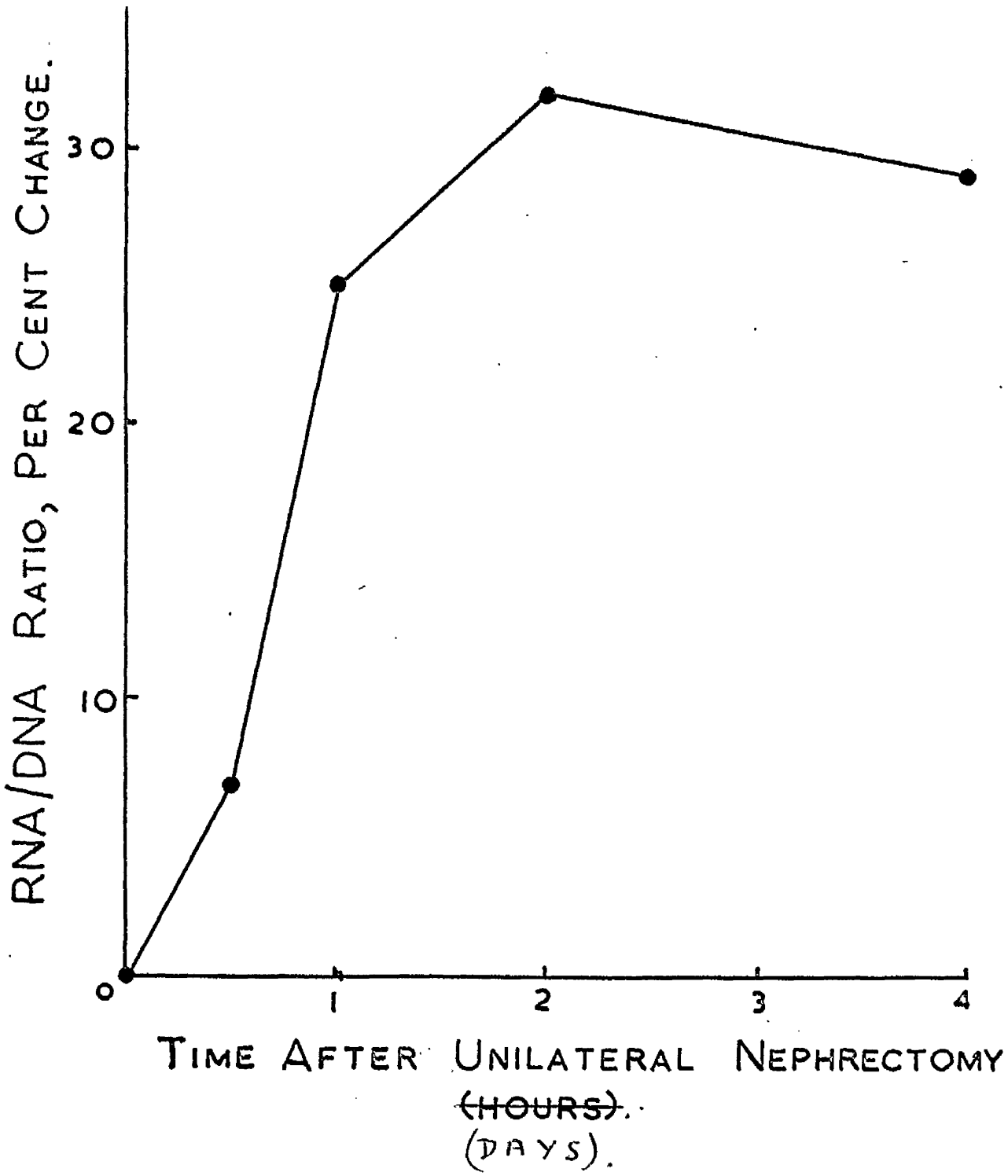
The changes in protein and nucleic acids in the surviving kidney have provided a means of estimating its growth after unilateral nephrectomy and of comparing this with the changes in normal kidney following various dietary additions. Nevertheless, these determinations give a measure of a change after it has occurred, rather than as it is occurring. Clearly in order to find the trigger to the compensatory growth, it will be necessary to study changes at very short time intervals after unilateral nephrectomy. The increases in kidney weight and in various chemical constituents develop too slowly to be detected with certainty before about 24 hours after the operation. So far the earliest chemical change which can be reliably demonstrated has been the increase in RNA per cell, which amounts to about 7% at 12 hours and 25% at 24 hours (Section 3.2.3.). Thus a gap of about 12 hours was separating the stimulus of unilateral nephrectomy from the first sign of hypertrophy in the surviving kidney. Something must be happening during this interval: some sequence of events must link stimulus and response. It would clearly be of great interest to establish the nature of at least some of these events. If the increase in RNA content represents an acceleration of RNA synthesis, it should be possible to demonstrate it by the use of isotopically labelled precursors, while it was still in progress.

In the selection of the precursor, several points had to be considered, not the least of which was the cost involved. Since all experiments would be *in vivo*, a larger amount of isotope would be required than for, say, tissue culture experiments. In addition, to allow for biological variation, experiments would have to be done at least in duplicate. The studies involved would tend, therefore, to be expensive in terms of isotope required. Although [^{14}C] formate incorporation has been used in the past to estimate RNA biosynthesis (Thomson, Ricceri and Peretta, 1960), and would be fairly inexpensive, it is unfortunately nonspecific and would label for example glycine and serine and thus protein and also all purines. It would therefore be necessary to isolate very pure RNA to be certain that the incorporation being measured was indeed incorporation into RNA. To avoid this, it seemed reasonable to use a purine or pyrimidine precursor which would be much more specific in labelling nucleic acids. The most suitable form of such a precursor would, in theory, be the triphosphate, since the enzyme synthesizing RNA utilises triphosphates, but unfortunately this again would be expensive and it is doubtful whether such a precursor would in fact get into the kidney cells without prior hydrolysis. The two free bases most extensively incorporated into ribonucleic acids in the rat are adenine and orotic acid (Brown and Roll, 1955). There was nothing to choose between these two, and adenine was selected. It was used in the tritiated form which is cheaper than the ^{14}C-labelled form.

Figure 28

The effect of right unilateral nephrectomy on the RNA/DNA ratio (ie. RNA per cell) of the remaining kidney, expressed as a percentage of the corresponding ratio for the kidney excised at operation. The points were derived from Tables 13, 21 and 24.

FIGURE 28.


RNA/DNA Ratio, Per Cent Change.
30
20
10
0
1
2
3
4
Time After Unilateral Nephrectomy
(~~Hours~~).
(Days).

It is clear from Figure 28, which is derived from Tables 13, 21 and 24, that changes in the RNA content per cell are significant at 12 hours post-operatively. Therefore in the period immediately preceeding this, RNA synthesis ought to be fairly rapid. Accordingly unilaterally nephrectomized and sham operated animals were injected intraperitoneally with 1 μc of $[^3H]$ adenine per gram of body weight 10 hours post-operatively and killed 2 hours later. The kidneys were homogenised. Duplicate samples were then analysed for incorporation of tritium as outlined in Section 2.2.4. In addition, duplicate samples were analysed for protein and nucleic acids in the usual manner. Table 42 shows the incorporation of the precursor in two separate experiments. The results obtained in the two experiments were very erratic. In the first experiment, the specific activity of the RNA was actually lower after unilateral nephrectomy than in sham operated animals. In the second experiment it was very much higher. In both experiments the radioactivity of the acid soluble material was estimated to obtain a rough indication of the specific activity of the free nucleotides from which RNA is synthesised. In the first experiment this acid soluble activity was about twice as high in the sham operated control as in the nephrectomized animal. This situation was almost exactly reversed in the second experiment. Table 43 shows the chemical analysis of the kidneys at the end of the experiment. There was no significant change in the RNA content per cell 12 hours post-operatively. Presumably this means that up to a point, there had been no measurable accumulation of RNA.

Table 42

The incorporation of [^{3}H] adenine into left kidney RNA after right unilateral nephrectomy or sham operation. The isotope, 1 μc./g. body weight, was injected 10 hours after the operation and the animals killed 2 hours later.

Experiment Number	Treatment	Specific activity of RNA (counts/min/μg. RNAP)	Specific activity of Acid Soluble Extract (counts/min/ 0.8 ml. extract)
1	Sham Operation	23.4	9,340
	Unilateral Nephrectomy	14.6	5,680
2	Sham Operation	0.6	2,565
	Unilateral Nephrectomy	12.4	4,355

The rats weighed 136-152 g. but were paired to within 5 g. for each experiment.

Table 43

The size and composition of the kidneys 12 hours after right unilateral nephrectomy or sham operation.

Experiment Number	Treatment	Kidney	Kidney Weight (mg.)	DNAP		RNAP		Protein	
				µg/100 mg. Wet Weight	µg/ kidney	µg/ kidney	µg/µg. DNAP	mg/ kidney	µg/µg. DNAP
1	Sham Operation	Right	576	31	180	476	1.52	84	467
		Left	552	39	217	565	1.47	99	457
	Unilateral Nephrectomy	Right	570	36	205	428	1.20	81	399
		Left	551	35	195	420	1.19	76	389
2	Sham Operation	Right	577	34	197	271	1.38	87	443
		Left	526	36	189	252	1.34	88	469
	Unilateral Nephrectomy	Right	546	36	196	253	1.29	95	482
		Left	495	35	171	230	1.35	79	463

The rats weighed 136 - 152 g. but were paired to within 5 g. for each experiment.

These rather discouraging results might, therefore, merely mean that RNA synthesis had not started as soon after unilateral nephrectomy as might have been expected. Accordingly a third experiment, similar to the first two, was performed, but the isotope was injected 18 hours post-operatively and the animals killed at 20 hours. To try to obtain higher activities, four times as much isotope as in the previous two experiments was used. Table 44 shows that there was about equal incorporation of the isotope into the RNA of sham operated and unilaterally nephrectomized animals. The activities of the acid soluble material in the two animals were also very similar. By this time however, there had clearly been an increase in the RNA content per cell of the remaining kidney of the operated animal (Table 45). So that the failure in this experiment to detect RNA synthesis isotopically could not be explained on the ground that there had been no accumulation of RNA. It seemed highly improbable, therefore, that this technique would reveal the sort of RNA synthesis which one might, on theoretical grounds, confidently predict.

It seemed possible that no great change in the uptake of isotope had been obtained in the previous three experiments because the pulse time of 2 hours which had been chosen, was too short. The adenosine-triphosphate (ATP) pool is very large (Keir, 1957). It is possible therefore, that the labelled adenine is incorporated, converted to ATP and enters the precursor pool where it is effectively diluted out by the unlabelled ATP already present. Accordingly, an alternative

Table 44

The incorporation of [^{3}H] adenine into left kidney RNA after right unilateral nephrectomy or sham operation. The isotope, 4 μc./g. body weight, was injected 18 hours after the operation and the animals killed 2 hours later.

Experiment Number	Treatment	Specific activity of RNA (counts/min/μg. RNAP)	Specific activity of Acid Soluble Extract (counts/min/ 0.8 ml. extract)
3	Sham Operation	99.8	2,482
	Unilateral Nephrectomy	111.2	2,676

The rats weighed 140 g. and 137 g. respectively.

Table 45

The size and composition of the kidneys 20 hours after right unilateral nephrectomy or sham operation.

Experiment Number	Treatment	Kidney	Kidney Weight (mg.)	DNAP		RNAP		Protein	
				µg/100 mg. Wet Weight	µg/ kidney	µg/ kidney	µg/µg. DNAP	mg/ kidney	µg/µg. DNAP
3	Sham Operation	Right	647	32	208	288	1.38	111	532
		Left	602	32	192	268	1.40	111	581
	Unilateral Nephrectomy	Right	572	37	213	272	1.28	105	493
		Left	568	34	195	287	1.47	104	533

The rats weighed 140 and 137 g. respectively.

type of experiment was carried out in which the isotope was injected at the time of operation. Twenty four hours later, the animals were killed. Each right kidney and one half of each left kidney was analysed as in the last three experiments. The results of this analysis are shown in Table 46. From the chemical analysis it is clear that the RNA content per cell of the remaining kidney in the operated animal increased. The uptake of isotope, however, was actually less than that of the sham operated animal. In addition, the activity of the acid soluble material of the operated animal was almost proportionally less than that of the sham operated animal. These findings are rather difficult to interpret. Not only must the precursor pool be diluting out the labelled ATP but the pool in the operated animal must also, for unknown reasons, be bigger than that in the control animal.

Whatever the cause may be, it is pretty clear that these attempts to estimate RNA biosynthesis by measuring the total incorporation of a labelled precursor into RNA were not likely to yield meaningful results. There could be a variety of causes of this. It might be that the purification procedure was inadequate and erratic in its effectiveness. Accordingly a different procedure was tried. As stated above in the last experiment (Table 46), only one half of each left kidney was analysed by the standard procedure. From the other half the RNA was extracted by the phenol method described in Section 2.5. and subjected to analysis on a sucrose density gradient

Table 46

The effect of right unilateral nephrectomy on RNA synthesis after 24 hours.

Treatment	Kidney	DNAP (µg/100 mg. Wet Weight)	RNAP (µg/100 mg. Wet Weight)	RNAP (µg/µg. DNAP)	counts/min/ µg. RNAP	counts/min/0.8 ml. Acid Soluble Fraction
Sham Operation	Right	29.7	42.8	1.44		
	Left	31.7	44.0	1.39	81.3	3272
Unilateral Nephrectomy	Right	32.2	43.2	1.34		
	Left	29.6	43.7	1.48	54.2	1903

The animals weighed 142 and 150 g. respectively. They were injected intraperitoneally with [^{3}H] adenine (2 µc/g. body weight) immediately after the operation and sacrificed 24 hours later.

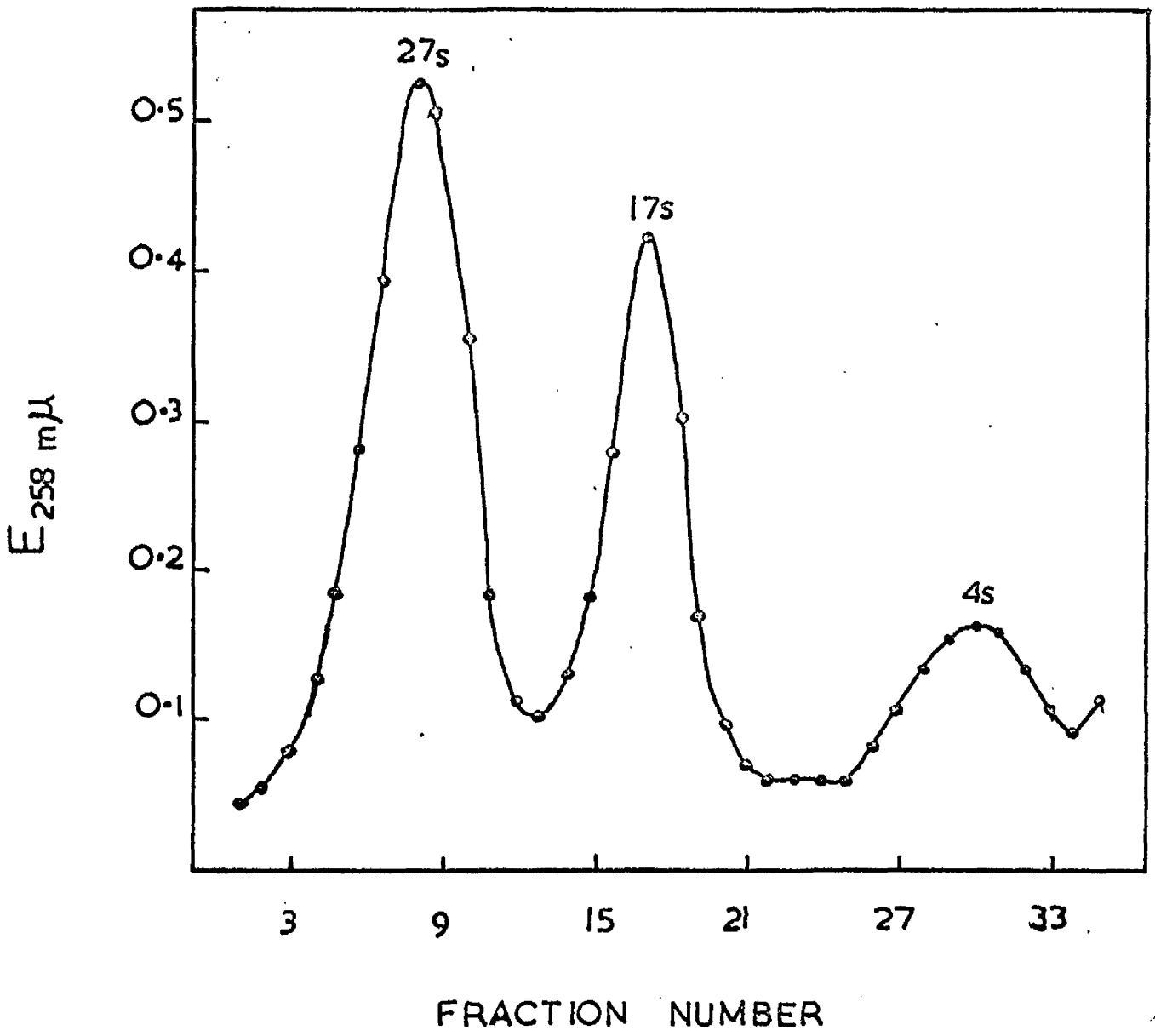
(Britten and Roberts, 1960). This technique permits the separation of RNA fractions which sediment at different rates when centrifuged. By puncturing the base of the tubes, after centrifugation, with a syringe needle, the separated RNA fractions can be collected in a series of tubes. The extinction (at 260 mμ) of each of these tubes is then obtained and plotted against tube number to give the type of profile shown in Figure 29. This extinction profile gives an indication of the total amount of RNA present. The material recovered from the bottom of the tube i.e. the higher molecular weight RNA, is at the left hand side of the figure. The two larger peaks represent ribosomal RNA and the third small peak, soluble RNA. If the RNA is also labelled, then by obtaining the radioactivity of each tube, another profile can be added, giving an indication of the amount of RNA synthesised during the time of exposure to the isotope. Thus the extinction profile will enable one to determine which fraction of RNA has become labelled, and the radioactivity profile will give an indication of the extent of labelling. By varying the pulse time, therefore, this procedure should make it possible to obtain pictures of the sequence in which the different RNA fractions are labelled.

The sedimentation coefficients of the two principal peaks of kidney RNA were calculated from their rates of sedimentation observed in the Spinco Model E Analytical Ultracentrifuge. The value for the fastest sedimenting peak was calculated to be 27.0s; that of the next fastest peak was calculated to be 16.8s. The slowest sedimenting

Figure 29

Sedimentation analysis in sucrose density gradients of RNA from rat kidney. The gradient was centrifuged at 23,000 rev./min. for 16 hr. in the SW 39 rotor.

FIGURE 29.



peak was assumed to have an s value of 4 by analogy with other workers (Hiatt, 1962; Luborsky and Cantoni, 1962; Scherrer and Darnell, 1962). In the subsequent paragraphs the three principal RNA peaks are referred to as 27s, 17s and 4s respectively. The corresponding values for liver RNA were calculated to be 28s, 18s and 4s. These s values for liver RNA are similar to those of Peterman and Pavlovec (1963) for whole liver RNA and to those of Hall and Doty (1959) for liver microsomal RNA.

When this technique was applied to the unilaterally nephrectomized and sham operated animals which had been injected with [^{3}H] adenine in the last experiment (Table 46), the results shown in Figure 30 were obtained. As can be seen, the radioactivity profiles paralleled the extinction profiles in both animals. In other words, the RNA was uniformly labelled in both cases. This is what one would have expected after a 24 hour 'pulse', because during this long time interval the isotope has time to be incorporated into all different species of RNA. The results of this experiment do, however, show that the isotope was actually being incorporated into RNA.

In order to detect any differences in the labelling of RNA fractions after unilateral nephrectomy, it was obviously necessary to use much shorter pulse times. Since the liver has been extensively examined in this way (Hiatt, 1962; Kidson, Kirby and Ralph, 1963; Di Girolamo, Di Girolamo, Gaetani and Spadoni, 1966), in subsequent experiments, RNA was extracted from the liver as well as the kidney

Figure 30

Sucrose density gradient analysis of RNA from kidneys of rats injected intraperitoneally with 2 μc [^{3}H] adenine per gram body weight 24 hours before death.

(a) Obtained from the left kidney of an animal subjected to right unilateral nephrectomy immediately before injection.

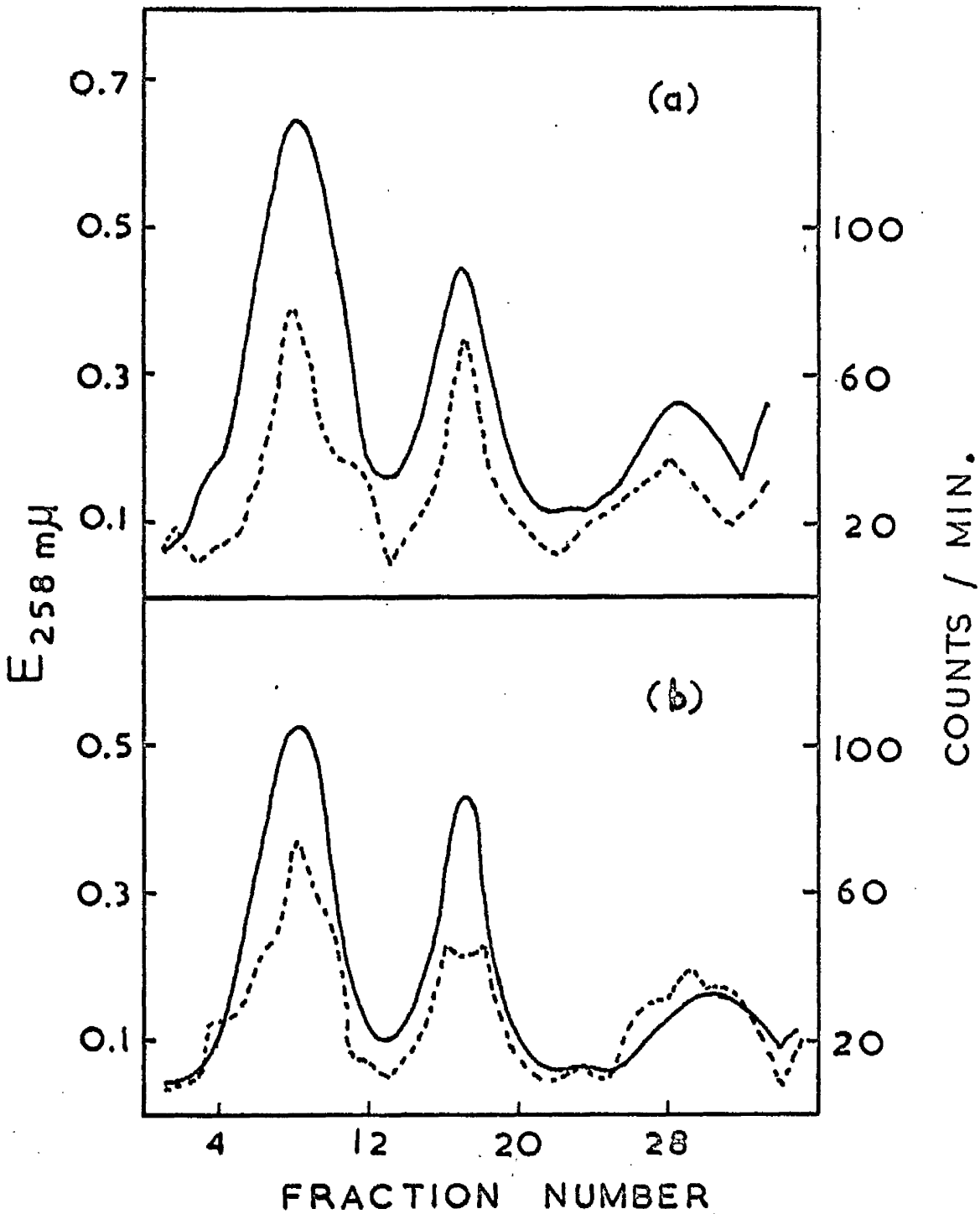
(b) Obtained from the left kidney of an animal subjected to right sham operation immediately before injection.

The gradients were centrifuged at 23,000 rev./min. for 16 hr. in the SW 39 rotor.

——— represents the $E_{258\ m\mu}$ of the collected fractions (diluted approximately 1 in 4).

- - - represents counts/min./0.8 ml. of the diluted fractions.

FIGURE 30.


(a)
(b)
0.7
0.5
0.3
0.1
0.5
0.3
0.1
100
60
20
100
60
20
$E_{258\,m\mu}$
COUNTS / MIN.
4
12
20
28
FRACTION NUMBER

to give a control picture and an indication of the validity and effectiveness of the method. In all cases the animals were injected with isotope immediately after the operation. Figure 31 shows the results obtained in an animal killed 6 hours later. The radioactivity profile for the liver RNA paralleled the extinction profile, indicating uniform labelling of all RNA fractions. There was no difference in the pattern of labelling in the liver RNA of sham operated and unilaterally nephrectomized animals. Similar results were obtained for kidney RNA, but the incorporation of isotope was very much less than that into liver RNA. Figure 32 shows the results in an animal killed 4 hours after the operation. In liver the same pattern of labelling was obtained as at 6 hours except that the incorporation was only about half as great. A very similar pattern of labelling has been obtained by Drysdale and Munro (1965) at this time interval using $[^{14}C]$ adenine as precursor. The pattern of labelling in the kidney, however, was quite different. The 4s RNA was extensively labelled but ribosomal RNA (27s and 17s) was not labelled to any degree at all. There was no difference in the pattern of labelling of kidney RNA in the two animals, but quantitatively the incorporation into the RNA of the sham operated animal was greater. Finally in an experiment in which the animals were killed 2 hours post-operatively there was practically no incorporation of isotope into kidney RNA of either the unilaterally nephrectomized animal or its sham operated control. Table 47 summarises the overall

Figure 31

Sucrose density gradient analysis of RNA from kidneys and livers of rats injected intraperitoneally with 2 μc $[^3H]$ adenine per gram body weight 6 hours before death.

(a) Obtained from the left kidney of an animal subjected to right unilateral nephrectomy immediately before injection.

(b) Obtained from the left kidney of an animal subjected to right sham operation immediately before injection.

(c) Obtained from the liver of the animal subjected to right unilateral nephrectomy.

(d) Obtained from the liver of the sham operated animal.

The gradients were centrifuged at 20,500 rev./min. for 16 hr. in the SW 39 rotor.

——— represents the $E_{258\ m\mu}$ of the collected fractions (diluted approximately 1 in 4).

- - - represents the counts/min./0.8 ml. of the diluted fractions.

FIGURE 31.

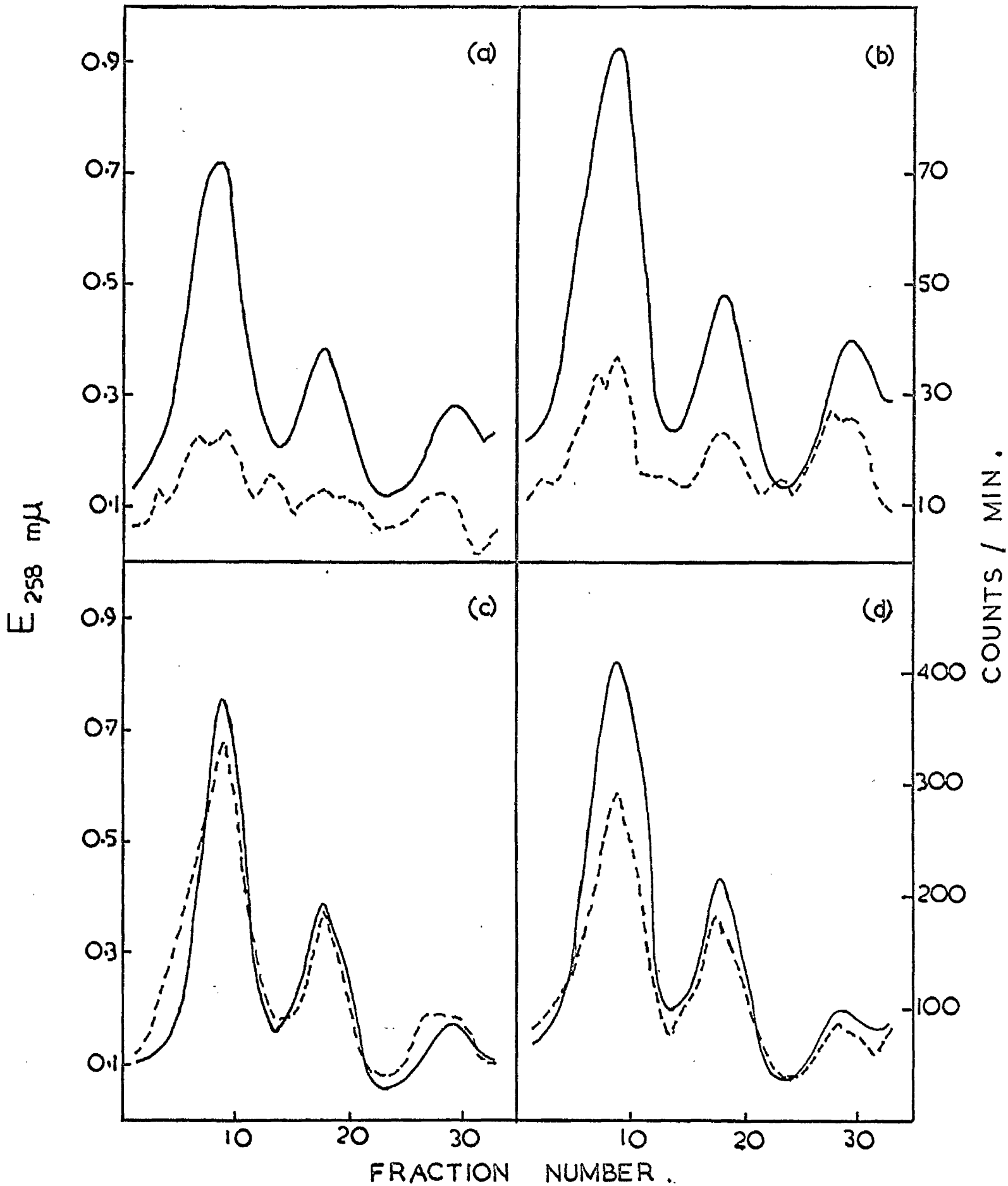

(a)
(b)
(c)
(d)
0·9
0·7
0·5
0·3
0·1
70
50
30
10
400
300
200
100
10
20
30
E_{258} mμ
COUNTS / MIN.
FRACTION NUMBER.

Figure 32

Sucrose density gradient analysis of RNA from kidneys and livers of rats injected intraperitoneally with 2 μc $[^3H]$ adenine per gram body weight 4 hours before death.

(a) Obtained from the left kidney of an animal subjected to right unilateral nephrectomy immediately before injection.

(b) Obtained from the left kidney of an animal subjected to right sham operation immediately before injection.

(c) Obtained from the liver of the animal subjected to right unilateral nephrectomy.

(d) Obtained from the liver of the sham operated animal.

The gradients were centrifuged at 20,500 rev./min. for 16 hr. in the SW 39 rotor.

——— represents the $E_{258\ m\mu}$ of the collected fractions (diluted approximately 1 in 4).

- - - represents counts/min./0.8 ml. of the diluted fractions.

FIGURE 32.

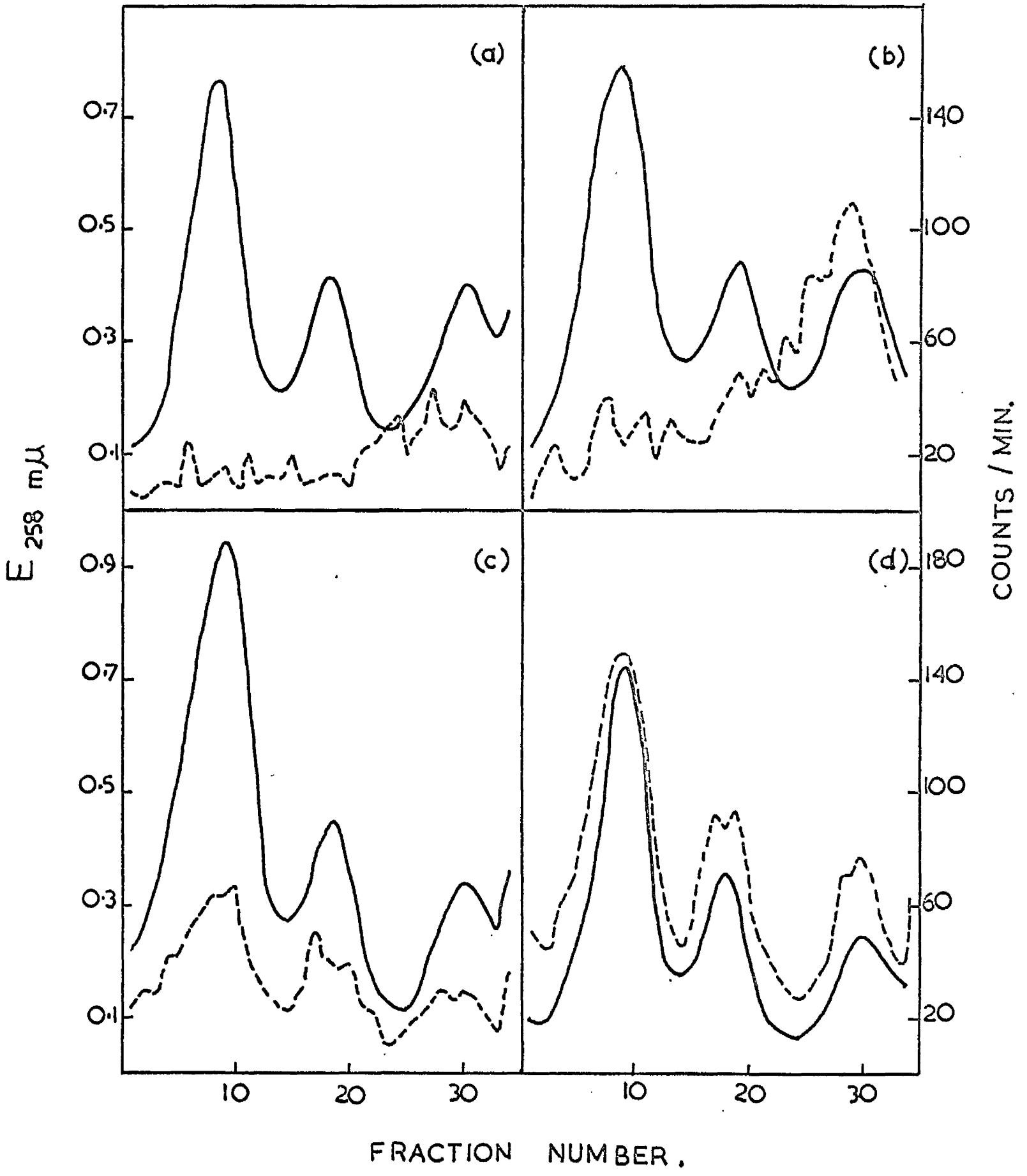

(a)
(b)
(c)
(d)
0·7
0·5
0·3
0·1
0·9
0·7
0·5
0·3
0·1
140
100
60
20
180
140
100
60
20
10
20
30
10
20
30
E_{258} mμ
COUNTS / MIN.
FRACTION NUMBER.

Table 47

Specific activities (counts/min./20 μg. RNAP) of kidney and liver RNA in unilaterally nephrectomized and in sham operated animals after injection of [^{3}H] adenine (2 μc./g. body weight).

Time Between Injection and Sacrifice (Hours)	Kidney		Liver	
	Unilaterally Nephrectomized Animal	Sham Operated Animal	Unilaterally Nephrectomized Animal	Sham Operated Animal
24	668	1080	-	-
6	224	248	1,442	1,150
4	118	287	598	752
2	66	59	-	-

The animals weighed between 130 and 150 g., but were matched to within 5 g. for each experiment. They were injected intraperitoneally with the isotope immediately after operation and were killed at the times shown.

specific activities of kidney and liver RNA following injection of [^{3}H] adenine. Clearly the figures for kidney show no consistent pattern. The [^{3}H] adenine experiments therefore failed to reveal any qualitative or quantitative difference in RNA metabolism after unilateral nephrectomy.

Since the incorporation of the adenine into kidney RNA (as opposed to liver RNA) was so poor, it seemed unlikely that further experiments using this precursor would serve any useful purpose. There was, however, the possibility that a different precursor might be more extensively incorporated. Accordingly 2 µc of [^{3}H] orotic acid per gram of body weight were injected intraperitoneally into unilaterally nephrectomized and control animals at the time of operation. Four hours later the animals were killed. As Table 48 shows, the incorporation of the isotope into kidney RNA was from 100 to 300 fold greater than the incorporation of [^{3}H] adenine in the same time period (Table 47). The incorporation into liver RNA was 24 to 30 fold greater. It would appear, therefore, that [^{3}H] orotic acid is a very much better precursor than [^{3}H] adenine for labelling kidney RNA. It is not clear why this should be so. Orotic acid of course will be incorporated into both the uracil and cytosine of the RNA, whereas adenine will have only one point of entry. Since the precursor pool sizes of UTP and CTP are very much smaller than that of ATP (Keir, 1957), the former two precursors will also be less extensively diluted out by unlabelled precursors than the latter. It is also possible

Table 48

Specific activities (counts/min/20 μg. RNAP) of kidney and liver RNA in unilaterally nephrectomized and in control animals at various times after injection of [^{3}H] orotic acid.

Isotope Injected per g. of Body Weight	Time Between Injection and Sacrifice	Animal	Specific Activity of RNA		
			Kidney	Liver	Ratio $\frac{\text{Kidney}}{\text{Liver}}$
2 μc.	4 Hours	Unilaterally Nephrectomized	32,500	18,100	1.80
		Sham Operated	-	-	-
		Intact Control	25,290	17,970	1.41
0.5 μc.	2 Hours	Unilaterally Nephrectomized	5,290	1,980	2.67
		Sham Operated	4,740	2,660	1.78
		Intact Control	4,230	3,260	1.30
0.5 μc.	70 Min.	Unilaterally Nephrectomized	5,100	1,800	2.83
		Sham Operated	5,620	3,860	1.46
		Intact Control	3,110	1,930	1.61
0.5 μc.	60 Min.	Unilaterally Nephrectomized	2,020	1,000	2.02
		Sham Operated	-	-	-
		Intact Control	2,830	2,250	1.26
1 μc.	20 Min.	Unilaterally Nephrectomized	5,070	1,270	3.99
		Sham Operated	-	-	-
		Intact Control	6,240	2,120	2.94

The animals weighed between 128 and 147 g., but were matched to within 5 g. for each experiment. They were injected intraperitoneally with the isotope immediately after the operation and were killed at the times shown.

that adenine may be broken down *in vivo* by adenase.

Figure 33 shows the sucrose density gradient results. There was uniform incorporation of the precursor into the RNA of kidney and liver. No qualitative differences between nephrectomized animals and controls were found in the patterns of labelling of kidney or liver RNA. Nor was any qualitative difference found in subsequent experiments in which the animals were killed 2 hours, 70 minutes or 20 minutes after the operation (Figures 34, 35 and 36 respectively). Table 48 summarises overall activities of kidney and liver RNA in these experiments. The absolute specific activites of the RNA varied considerably. Generally speaking the figures obtained for the surviving kidney of unilaterally nephrectomized animals were not very different from those given by intact or sham operated controls. The same is true of the observed specific activities in the liver. If, however, the ratio of the specific activity of kidney RNA to that of liver RNA was calculated, a small but consistent difference became apparent. In each of the five experiments the ratio obtained for the nephrectomized animal was 27% to 119% greater than the corresponding ratio for the controls. This finding, which was surprising after so many negative results, obviously required corroboration. Table 49 shows the results of four confirmatory experiments, in all of which the animals were killed two hours after the operation. Once again the ratios of specific activity were consistently higher in nephrectomized animals than in controls. This represents one of the

Figure 53

Sucrose density gradient analysis of RNA from kidneys and livers of rats injected intraperitoneally with 2 μc [^{3}H] orotic acid per gram body weight 4 hours before death.

(a) Obtained from the left kidney of an animal subjected to right unilateral nephrectomy immediately before injection.

(b) Obtained from the left kidney of an unoperated control animal.

(c) Obtained from the liver of the animal subjected to right unilateral nephrectomy.

(d) Obtained from the liver of the unoperated control animal.

The gradients were centrifuged at 20,500 rev./min. for 16 hr. in the SW 39 rotor.

——— represents the $E_{250\ m\mu}$ of the collected fractions (diluted approximately 1 in 4).

– – – represents counts/min./0.8 ml. of the diluted fractions.

FIGURE 33.

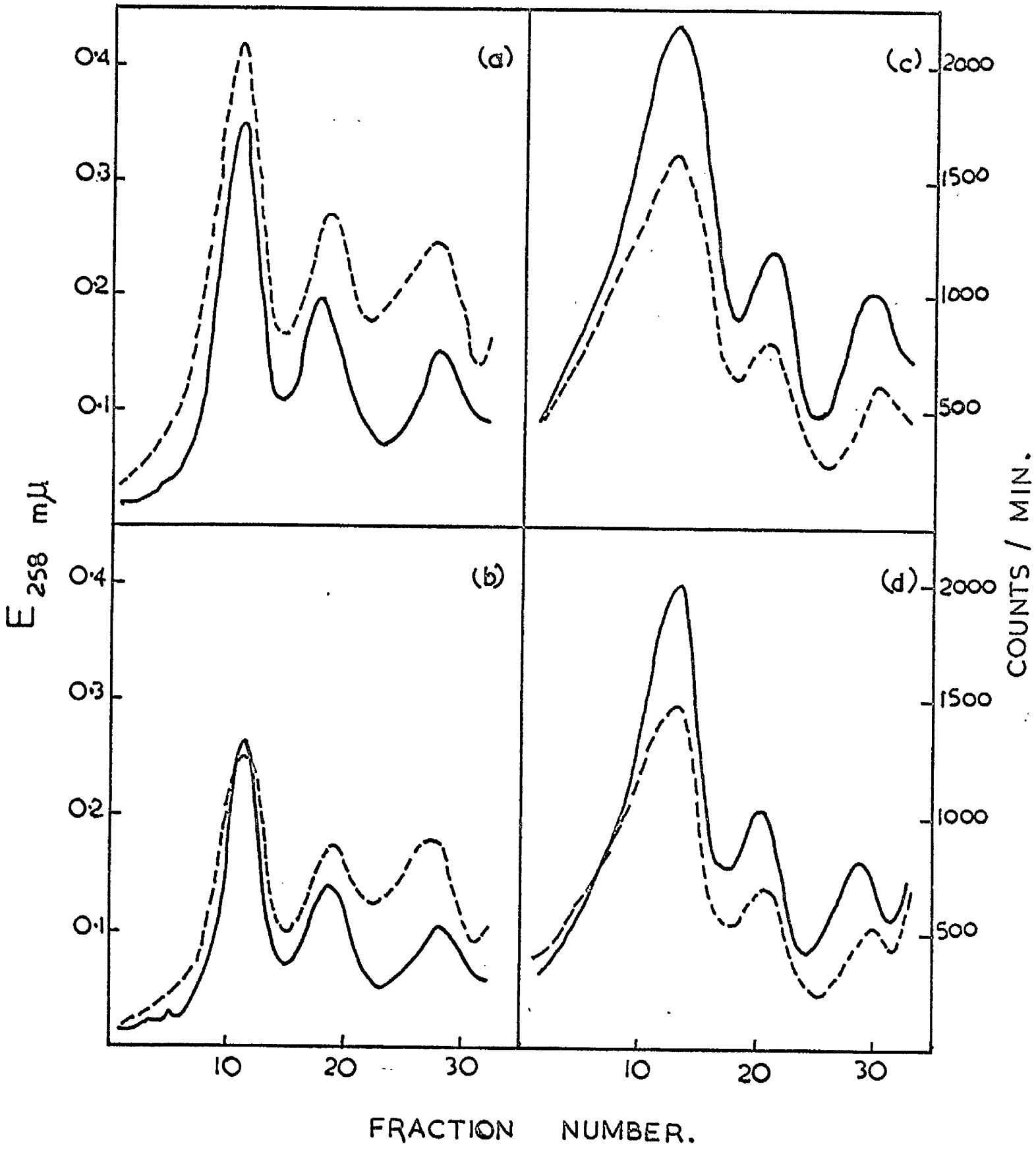

(a)
(b)
(c)
(d)
0·4
0·3
0·2
0·1
2000
1500
1000
500
10
20
30
E_{258} mμ
COUNTS / MIN.
FRACTION NUMBER.

Figure 34

Sucrose density gradient analysis of RNA from kidneys and livers of rats injected with 0.5 μc [^{3}H] orotic acid per gram body weight 2 hours before death.

(a) Obtained from the left kidney of an animal subjected to right unilateral nephrectomy immediately before injection.

(b) Obtained from the left kidney of an animal subjected to right sham operation immediately before injection.

(c) Obtained from the left kidney of an unoperated control animal.

(d) Obtained from the liver of the unilaterally nephrectomized animal.

(e) Obtained from the liver of the sham operated animal.

(f) Obtained from the liver of the unoperated control animal.

The gradients were centrifuged at 39,000 rev./min. for 2 hr. 50 min. in the SW 39 rotor.

——— represents the $E_{258\ m\mu}$ of the collected fractions (diluted approximately 1 in 4).

- - - represents counts/min./0.8 ml. of the diluted fractions.

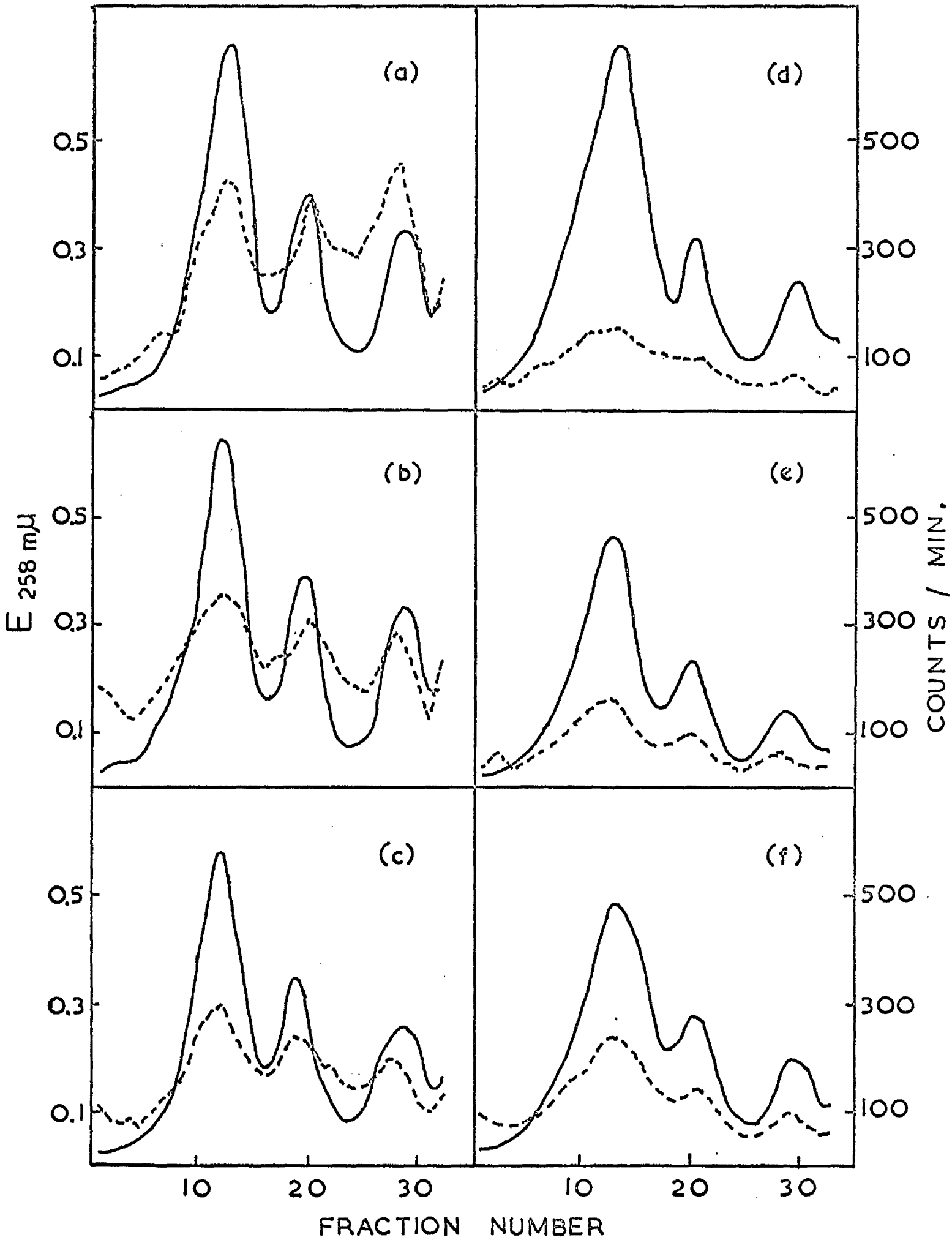

(a)
(b)
(c)
(d)
(e)
(f)
0.5
0.3
0.1
500
300
100
10
20
30
E 258 mμ
COUNTS / MIN.
FRACTION NUMBER

Figure 35

Sucrose density gradient analysis of RNA from kidneys and livers of rats injected with 0.5 μc [^{3}H] orotic acid per gram body weight 70 min. before death.

(a) Obtained from the left kidney of an animal subjected to right unilateral nephrectomy immediately before injection.

(b) Obtained from the left kidney of an animal subjected to right sham operation immediately before injection.

(c) Obtained from the left kidney of an unoperated control animal.

(d) Obtained from the liver of the unilaterally nephrectomized animal.

(e) Obtained from the liver of the sham operated animal.

(f) Obtained from the liver of the unoperated control animal.

The gradients were centrifuged at 39,000 rev./min. for 2 hr. 50 min. in the SW 39 rotor.

——— represents the $E_{258\ m\mu}$ of the collected fractions (diluted approximately 1 in 4).

- - - represents counts/min./0.8 ml. of the diluted fractions.

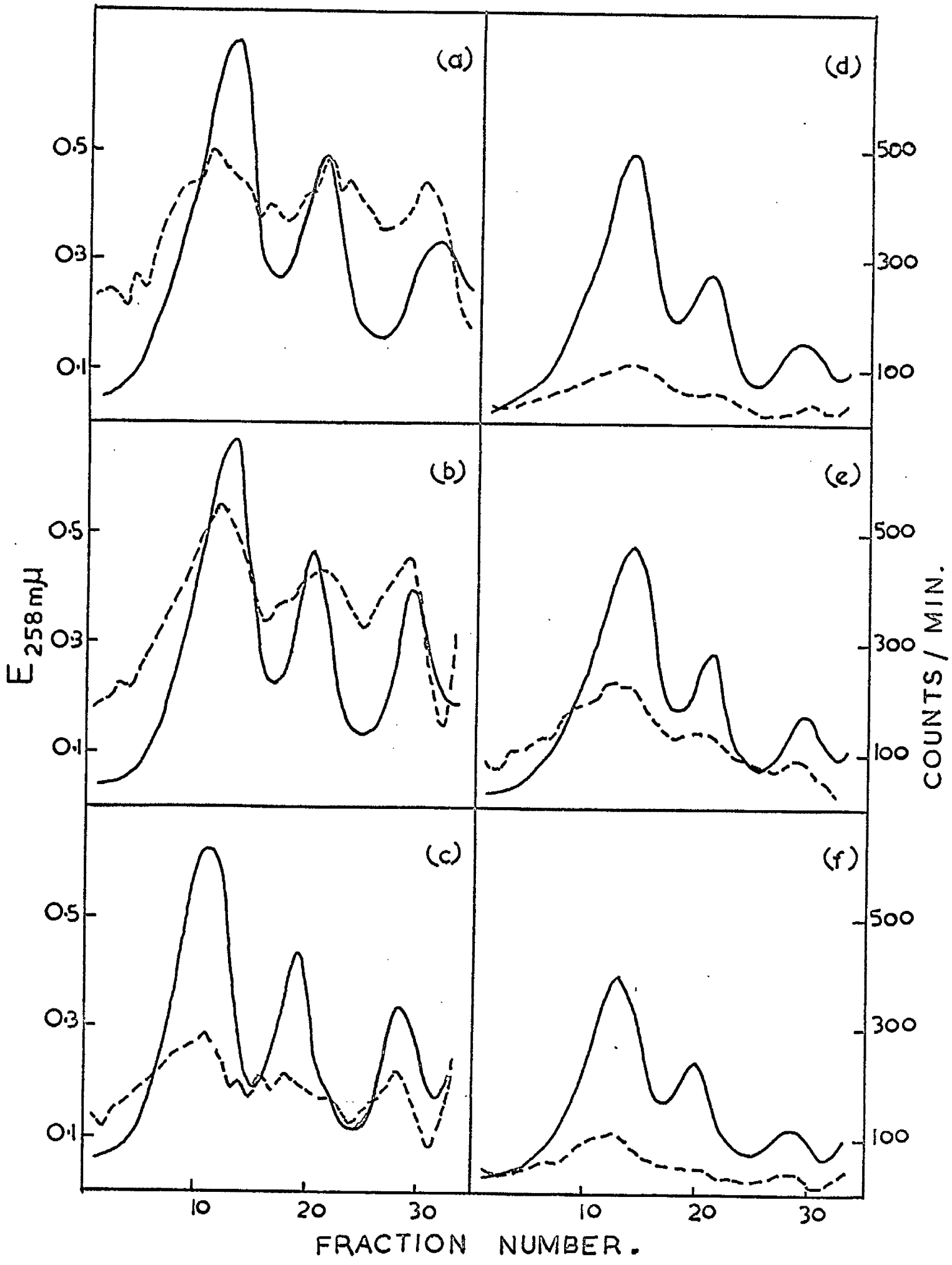

(a)
(b)
(c)
(d)
(e)
(f)
0·5
0·3
0·1
500
300
100
10
20
30
E 258 mμ
COUNTS / MIN.
FRACTION NUMBER.

Figure 36

Sucrose density gradient analysis of RNA from kidneys and livers of rats injected with 1 μc $[^{3}H]$ orotic acid per gram body weight 20 min. before death.

(a) Obtained from the left kidney of an animal subjected to right unilateral nephrectomy immediately before injection.

(b) Obtained from the left kidney of an unoperated control animal.

(c) Obtained from the liver of the unilaterally nephrectomized animal.

(d) Obtained from the liver of the unoperated control animal.

The gradients were centrifuged at 39,000 rev./min. for 2 hr. 40 min. in the SW 39 rotor.

——— represents the $E_{258\ m\mu}$ of the collected fractions (diluted approximately 1 in 4).

– – – represents counts/min./0.8 ml. of the diluted fractions.

FIGURE 36.

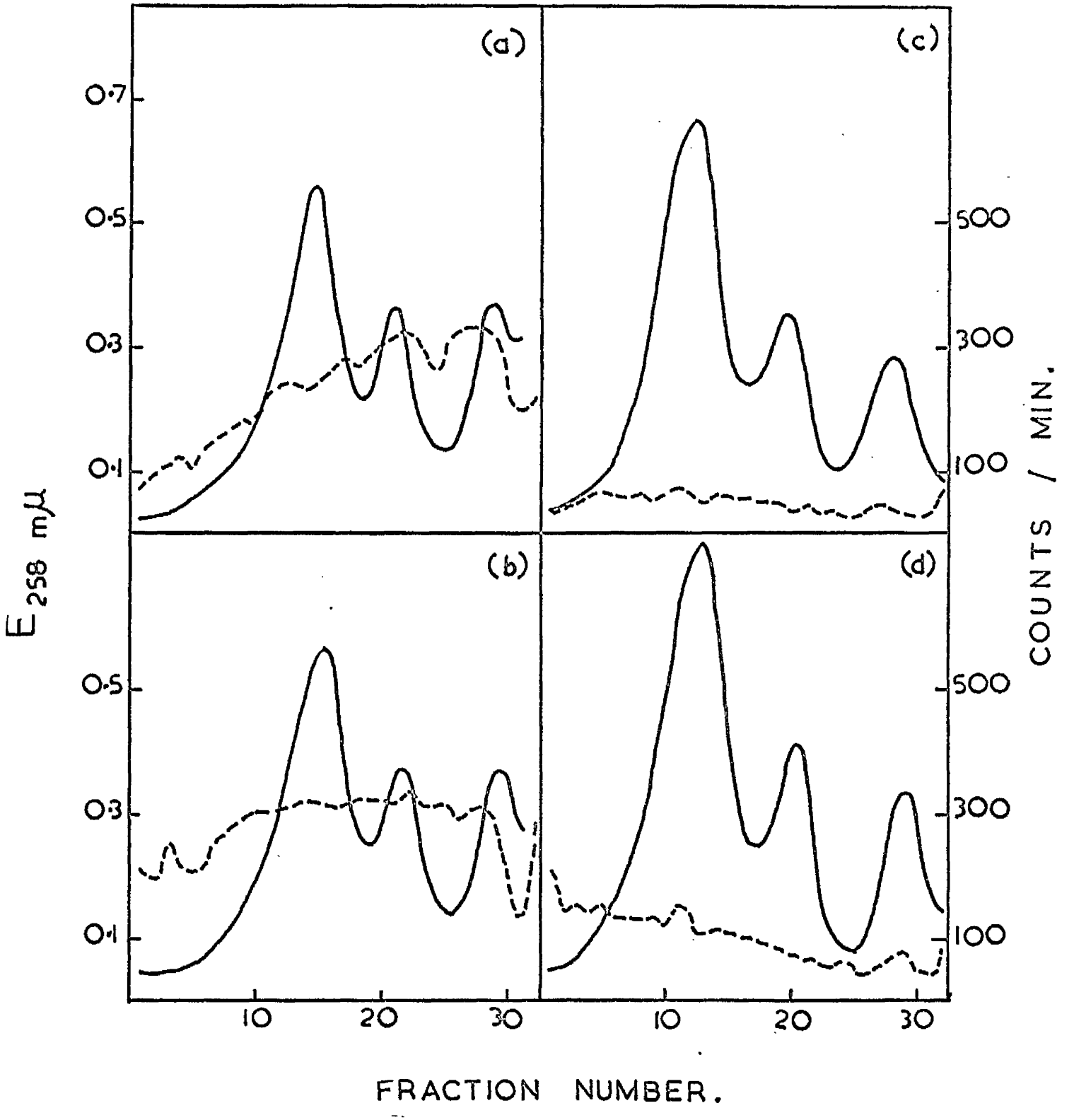

(a)
(c)
(b)
(d)
0·7
0·5
0·3
0·1
500
300
100
10
20
30
E_{258} mμ
COUNTS / MIN.
FRACTION NUMBER.

Table 49

Specific activities (counts/min/20 μg. RNAP) of kidney and liver RNA of unilaterally nephrectomized and control animals 2 hours after injection of [^{3}H] orotic acid (0.5 μc. / g. body weight.)

Animal	Specific Activity of RNA		
	Kidney	Liver	Ratio $\frac{\text{Kidney}}{\text{Liver}}$
Unilaterally Nephrectomized	5,120	2,730	1.88
Sham Operated	3,630	3,190	1.14
Intact Control	-	-	-
Unilaterally Nephrectomized	10,700	3,230	3.31
Sham Operated	-	-	-
Intact Control	7,200	4,770	1.51
Unilaterally Nephrectomized	4,600	2,350	1.96
Sham Operated	4,000	3,550	1.13
Intact Control	-	-	-
Unilaterally Nephrectomized	4,160	2,360	1.76
Sham Operated	3,960	3,840	1.03
Intact Control	-	-	-

The animals weighed between 117 and 140 g. but were matched to within 5 g. for each experiment. They were injected intraperitoneally with the isotope immediately after the operation and were killed 2 hours later.

earliest changes ever observed in the surviving kidney after unilateral nephrectomy. What the mechanism may be is a matter for speculation. At first sight it suggests that unilateral nephrectomy increases the rate of RNA synthesis in the surviving kidney relative to RNA synthesis in the liver. Presumably the effect of the nephrectomy is to accelerate RNA synthesis in the surviving kidney but not in the liver. In order to check whether RNA synthesis in the liver is affected by unilateral nephrectomy, a group of animals was unilaterally nephrectomized and at the same time a small biopsy sample of liver was removed. The sample removed was the left radicle of the median lobe. In 6 normal animals this amounted to 10.2 $\pm$ 1.08% (i.e. mean $\pm$ standard deviation) of the total liver weight. Forty eight hours after the operation, the animals were sacrificed and the remaining kidneys and the larger of the right lateral lobes of the liver removed. In the 6 normal animals this lobe amounted to 14.7 $\pm$ 1.35% of the total liver weight. Table 50 shows the effect of the unilateral nephrectomy on the composition of the remaining kidney and the liver. The response of the surviving kidney was exactly as expected from past results (Tables 18 and 20), with an increase in mean cell mass and RNA and protein contents per cell. Exactly analagous changes were, however, found in the liver. It was obviously essential to discover whether these changes in the liver composition were a response specifically to the unilateral nephrectomy. It was possible that the differences found were due to differences in the composition of the different lobes of

Table 50

The effect of right unilateral nephrectomy on kidney and liver composition after 48 hours.

Tissue	Time of Removal (Hours)	DNAP µg/100 mg. Wet Weight	RNAP µg/µg. DNAP	Protein µg/µg. DNAP
Right Kidney	0	37.4 ± 1.27	1.33 ± 0.030	460 ± 23.6
Left Kidney	48	33.9 ± 0.73	1.64 ± 0.030	516 ± 22.0
Ratio $\frac{\text{Left}}{\text{Right}}$		0.91 ± 0.020	1.23 ± 0.024	1.13 ± 0.027
Liver Biopsy (1)	0	22.9 ± 0.49	3.98 ± 0.110	845 ± 25.0
Liver Biopsy (2)	48	20.4 ± 0.554	4.78 ± 0.094	909 ± 17.4
Ratio $\frac{(2)}{(1)}$		0.89 ± 0.028	1.21 ± 0.031	1.08 ± 0.035

Values are means ± S.E.M. for six animals weighing 144-155 g. They were subjected to right unilateral nephrectomy and removal of liver biopsy 1 (the left radicle of the median lobe). They were killed 48 hours later and the remaining kidney and liver biopsy 2 (larger of the right lateral lobes) analysed.

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]
[illegible]	[illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]
[illegible]		[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]
(C) [illegible]	[illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]
( ) [illegible]	[illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]
[illegible]		[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]

[illegible] ± [illegible]

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[illegible]

([illegible])

the liver. This possibility was examined by analysing chemically the median, right lateral and left lateral lobes of the livers of normal animals. The results are presented in Table 51. The concentrations of RNA and DNA did not vary from one lobe to another. This explanation is therefore ruled out. Alternatively the mere process of removing a biopsy may provoke a reaction in the remainder of the liver. Fujioka, Koga and Lieberman (1963) have shown that a threshold amount of liver tissue, corresponding to 10% by weight of the total organ, must be removed before an increased incorporation of precursors into liver RNA is observed. MacDonald, Rogers and Pechet (1962) have reported that 9.4 to 12.3% by weight of liver tissue must be removed to effect a significant stimulation of DNA synthesis. Bucher and Swaffield (1964) have obtained similar results following removal of 9% of the liver. The amount of liver tissue being removed in the present experiment (about 10%) is very comparable to that removed by these three groups of workers. It was possible, therefore, that the removal of the liver biopsy, and not the unilateral nephrectomy, was the cause of the changes in liver composition. Accordingly the biopsy sample of liver was removed from another group of animals which were otherwise left intact. After 48 hours the animals were killed and the right lateral lobes of their livers analysed. The results are shown in Table 52. There had been increases in mean cell mass and in the RNA and protein contents per cell similar to those found in the

Table 51

Comparison of DNAP and RNAP content of median, right lateral and left lateral lobes of rat liver.

Liver Lobe	DNAP µg/100 mg. wet weight	RNAP µg/100 mg. wet weight	$\frac{\text{RNAP}}{\text{DNAP}}$
Median	22.6	87.6	3.87
Right Lateral	22.2	86.8	3.93
Left Lateral	22.2	85.9	3.90

Values are means for 3 animals weighing 141 to 152 grams.

Table 52

The effect of removal of a liver biopsy on liver composition after 48 hours.

Tissue	Time of Removal (Hours)	DNAP µg/100 mg. Wet Weight	RNAP µg/µg. DNAP	Protein µg/µg. DNAP
Liver Biopsy (1)	0	21.5 ± 0.38	4.00 ± 0.046	884 ± 34.1
Liver Biopsy (2)	48	19.0 ± 0.39	4.70 ± 0.111	945 ± 44.7
Ratio $\frac{(2)}{(1)}$		0.92 ± 0.023	1.18 ± 0.028	1.08 ± 0.076

Values are means ± S.E.M. for 5 animals weighing 138-143 g. At operation, the left radicle of the median lobe was removed (liver biopsy 1). 48 hours later the animals were sacrificed and the larger of the right lateral lobes removed (liver biopsy 2).

nephrectomy experiment (Table 50). These changes were, therefore, simply a result of the liver biopsy and presumably had nothing to do with the unilateral nephrectomy.

The changes in liver composition seemed to be large in relation to the small amount of liver tissue removed. It has long been assumed by physiologists that the liver has a large reserve capacity, but if it responds so markedly to the loss of 10% of its tissue, this can scarcely be the case. It is possible that the changes in the composition of the liver in the last two experiments were due to responses to stress of the animals. Until used for the experiment, the animals were, from birth, communally caged, each cage containing twelve animals. For the purpose of the experiment, they were transferred to a different room, in which the hours of electric light were different, and caged individually. In case the stress of this transfer had an effect on liver composition, another group of animals was transferred to this experimental room, caged individually, and fed and watered ad libitum for 10 days before removal of the liver biopsy. After a further 48 hours, they were killed. The remaining liver tissue again showed increases in mean cell mass and RNA and protein contents per cell (Table 53) similar to those in the preceeding experiment. It is clear, therefore, that unilateral nephrectomy itself does not markedly affect the liver content of RNA and it would appear that the ratio of the specific activity of the kidney RNA to that of the liver RNA after injection of [^{3}H] orotic acid

Table 53

The effect of caging the animals individually for 10 days, prior to removing a liver biopsy, on the liver composition.

Tissue	Time of Removal (Hours)	DNAP µg/100 mg. Wet Weight	RNAP µg/µg. DNAP	Protein µg/µg. DNAP
Liver Biopsy (1)	0	21.0 ± 0.63	4.20 ± 0.080	865 ± 28.8
Liver Biopsy (2)	48	19.9 ± 0.45	4.94 ± 0.072	933 ± 17.2
Ratio $\frac{(2)}{(1)}$		0.95 ± 0.001	1.18 ± 0.020	1.08 ± 0.023

Values are means ± S.E.M. for six animals weighing 116-155 g. The procedure in this experiment was identical with the experiment shown in Table 52, except that instead of being kept in communal cages before operation and in individual cages thereafter, the animals were transferred to individual cages 10 days before the operation and kept in individual cages until death.

gives early evidence of the process of compensatory renal hypertrophy.

Section 4

D I S C U S S I O N

D I S C U S S I O N

Conclusions

The purpose of the present investigation, as set out in the Introduction (Section 1.5.), was to apply chemical methods to an old problem which had so far defied attack by other routes. It should be stressed that the experiments are necessarily preliminary in character. The problem of compensatory renal hypertrophy has not so far attracted the attention of biochemists. The present work cannot be claimed to be more than a preliminary reconnaissance of a field which, sooner or later, will require a detailed survey. Nevertheless, it does demonstrate that the application of simple biochemical methods to a problem of this sort can yield substantial dividends. In the present instance, the use of DNA as a measure of cell number has shown that although the two kidneys of a single animal may (and generally do) differ in weight, they are almost identical in terms of cell size and composition. This has meant that the process of compensatory renal hypertrophy can be followed, much more precisely than hitherto, by observing the cell size and composition in the surviving kidney, and comparing them with the corresponding figures for its excised partner. By this means it has been possible to discern a chemical pattern in compensatory renal hypertrophy. This is characterized by a premitotic phase in which there is an initial increase in RNA per cell followed by smaller increases in mean cell mass and protein content. At about

36 hours, mitosis starts, resulting in a slow increase in cell number, while the mean cell mass and protein content return toward normal, though RNA per cell remains high.

Comparable chemical changes have been shown, by other workers, to occur in the remaining fragment of the liver after partial hepatectomy. Thus an increase in RNA per cell has been reported as early as 12 to 15 hours after the operation, rising to 40 to 60% at 24 hours (Price and Laird, 1950; Ultmann, Hirschberg and Gellhorn, 1953). Increases in protein content have been reported after 18 to 24 hours (Price and Laird, 1950; Tsuboi, Yokoyama, Stowell and Wilson, 1954; Harkness, 1957). These changes finally culminate in cell division 24 to 30 hours after the operation (Harkness, 1957).

Compensatory renal growth after unilateral nephrectomy involves mainly an hypertrophy and to a much lesser extent an hyperplasia (Simpson, 1961b). Compensatory growth of the liver after partial hepatectomy involves mainly an hyperplasia and to a much smaller extent, an hypertrophy (Harkness, 1957). Erythropoiesis, on the other hand, involves entirely an increase in the rate of division of precursor cells i.e. pure hyperplasia (Linman and Bethell, 1960). Thus there are obvious differences in the compensatory growth responses of even these three tissues. Nevertheless, comparative data relating to a number of tissues and organs might reveal, as in the case above of compensatory renal hypertrophy and liver regeneration, many similarities and perhaps a number of interesting

contrasts. It does seem clear, however, that the chemical investigation of compensatory renal hypertrophy is likely to bring us closer to understanding the mechanism controlling it than the traditional methods have done. From the chemical point of view, the problem of compensatory renal hypertrophy can profitably be subdivided into two specific questions :

1. What is the nature of the stimulus (chemical or otherwise) which, after unilateral nephrectomy, provokes the surviving kidney to hypertrophy?

2. By what chemical mechanism does this stimulus produce its effects?

It will be convenient to consider these questions separately.

1. The stimulus to compensatory renal hypertrophy

Compensatory renal hypertrophy is not by any means a unique occurrence. It is a general fact that when an organ of the body is damaged, it undergoes repair. An obvious example is wound healing, but this is too complex mechanically for easy investigation. It is rather easier to investigate situations where part of a tissue or organ can be removed with a minimum of trauma to the remainder. The compensatory growth which often follows such a loss of tissue can then be followed fairly easily. Examples of such a process are the increased erythropoiesis which follows extensive haemorrhage, liver regeneration after partial hepatectomy or compensatory renal hypertrophy following unilateral nephrectomy.

It is rather tempting to suppose that the amount of all these tissues is determined primarily by the work they have to do; the more work they are called upon to perform, the larger they grow. There are many examples of situations in which tissues or organs grow in response to increased functional demands. The hypertrophy of muscles when called upon to work harder (Goss, 1964a), of the heart in hypertension (Goss, 1964b), increased erythropoiesis under conditions of hypoxia (Gordon, Winkert, Dornfest, Lo Bue and Crusco, 1959; Fisher, Sanzari, Birdwell and Crook, 1962) and hyperplasia in lymphatic organs when challenged antigenically (Leduc, Coons and Connolly, 1955) are all examples of compensatory growth in response to increased functional demands. In point of fact, there are few tissues that will not enlarge when called upon to work harder.

Only in the case of erythropoiesis, however, is the mechanism of this work hypertrophy known. The compensatory production of red blood cells can be induced experimentally by excessive loss or destruction of red blood corpuscles (Hodgson and Tohá, 1954; Erslev, 1959). A similar acceleration of erythropoiesis will follow hypoxia or exposure to high altitudes (Hurtado, Merino and Delgado, 1945; Huff, Lawrence, Sixi, Wasserman and Hennesy, 1951; Fisher, Schofield and Porteus, 1965). Erythropoiesis, therefore, is stimulated when the demand for oxygen by tissues exceeds the supply. The respiratory requirements are communicated to the erythropoietic centres by erythropoietin, a compound believed to be

manufactured in the kidneys and carried by the blood to the marrow where it stimulates erythropoiesis (Jacobson, Goldwasser, Fried and Plazak, 1957; Naets, 1958; Erslev, 1960; Fisher et al., 1965). It exerts its effects on the earlier phases of erythropoiesis by increasing the numbers of young nucleated red cells capable of haemoglobin synthesis, without affecting the differentiation of later stages (Erslev, 1959; Filmanowicz and Gurney, 1959; Gallien-Lartigue and Goldwasser, 1965). Apart from more commonly recognised hormones, erythropoietin is the only accepted example of a growth controlling compound normally occurring in the blood. As such, its chemical nature is of considerable interest. The evidence available indicates that erythropoietin is partly proteinaceous and partly carbohydrate in nature. Thus it is precipitated by 75% saturated ammonium sulphate and migrates with the α_2 -globulins in electrophoresis (Rambach, Alt and Cooper, 1957); it absorbs ultra violet light at 280 mμ (Gordon et al., 1959) and its activity is abolished by digestion with pepsin, trypsin or chymotrypsin (Slaunwhite, Mirand and Prentice, 1957; Gordon et al., 1959). In addition, Rambach et al. (1957) have demonstrated that erythropoietin contains nitrogen and stains for carbohydrate, and Gordon et al. (1959) have shown that it is approximately 25% carbohydrate. Thus the evidence indicates that erythropoietin may be classified as a glycoprotein. In view of the relatively advanced state of the knowledge in this specific field, the erythropoietic regulatory system may well serve

as a model upon which to base hypotheses concerning growth regulation in general.

The mechanism controlling liver regeneration is much less clear. The existence of tissue specific growth controlling factors in the blood stream has been suspected for a long time. Only recently has conclusive evidence for the presence of such factors been demonstrated by the experiments of Leong, Grisham, Hole and Albright (1964). These workers transplanted the median lobe of the rat's liver to the abdominal subcutaneous tissue by a two stage procedure. Stage one involved transfer of a pedicle of the median lobe through a midline abdominal incision to the subcutaneous position. Stage two, performed two weeks later, involved ligation with a steel ligature of the pedicle and its blood vessels but excluding the bile drainage pathway. This procedure, therefore, left the autograft dependent on a collateral blood supply from the subcutaneous tissues but preserved its normal bile drainage. When the main part of the liver was partially hepatectomized 1-3 months later, DNA synthesis (as measured by uptake of $[^3H]$ thymidine) and mitosis occurred in the graft as well as in the residual liver. Thus these experiments conclusively demonstrated that the stimulus to regeneration is not local, but systemic. Whether it is mediated by a specific growth controlling hormone analagous to erythropoietin, or via a work hypertrophy effect due to an increase in the blood level of metabolites, is not clear and as Leong et al. pointed out, their experiments did

not provide any information concerning the nature, origin or mode of action of the blood-borne stimulator of liver regeneration.

On the other hand, attempts to demonstrate a specific hormone by parabiosis of a partially hepatectomized and an unoperated rat (Rogers, Shaka, Pechet and MacDonald, 1961) or by cross-circulation of the same combination of animals (Alston and Thomson, 1963), have been completely unsuccessful. Thus the stimulus to liver regeneration cannot be transferred from one animal to another. There is, therefore, no stable liver hormone analagous to erythropoietin. The stimulus to regeneration may therefore be a functional overload. In other words the remaining fragment may be regenerating in response to some sort of hepatic insufficiency. This idea is unfortunately difficult to test since the liver has many functions, and not a single well-defined one like the erythrocytes. The same problem has been encountered with compensatory renal hypertrophy. A number of workers have attempted to get round this problem by trying to devise techniques which increase all the functions which the kidneys of normal rats must do. These have included injection of urine, severing of one of the ureters and transplanting one of the ureters into the small intestine. In all of these cases the urine must be reabsorbed and re-excreted. These techniques should, therefore, increase all the kidney functions at the one time. In theory, such experiments are beyond criticism. In practice, however, there are grave technical difficulties (See section 3.3.) and no clear cut

answer has been obtained.

The present investigation of the control of compensatory renal hypertrophy has employed the alternative approach of increasing the kidney functions selectively, by feeding excess of materials to be excreted via the kidneys. In this way it has been shown that neither urea excretion (Section 3.3.3.) nor excretion of sodium ions or chloride ions or of water (Section 3.3.4.) has any marked effect on kidney size. Following high-protein diets (Section 3.3.1.) or acidosis (Section 3.3.4.), however, there is a highly significant increase in kidney size and composition. As shown in Table 40, however, the changes in kidney size and composition produced by these variations in the diet do not exactly parallel the changes in the remaining kidney after unilateral nephrectomy; in the former case, there is no increase in cell number, as reflected in the total DNA content, and the increase in RNA content per cell is roughly equal to the increase in protein content per cell. In the latter case, on the other hand, there is a small but detectable increase in cell number and the increase in RNA content per cell is generally very much greater than the increase in protein content per cell. In other words, the growth of the kidneys in response to variations in the diet is not exactly the same as the growth of the remaining kidney after unilateral nephrectomy. When considered together with the results of the ureteric transplantation experiments mentioned above, it would seem that the growth of the remaining kidney after

unilateral nephrectomy cannot be entirely explained in terms of the increased work it is called upon to perform. There seems little doubt that "work hypertrophy" of the kidney can and probably does occur following unilateral nephrectomy, but it seems unlikely that this is the mechanism initiating and controlling compensatory renal hypertrophy.

2. Early chemical changes in compensatory renal hypertrophy.

In our present uncertainties about the control of compensatory renal hypertrophy, a new approach to the problem is badly needed. It seems likely that the solution must be sought by looking for changes at earlier time intervals after unilateral nephrectomy. Since compensatory renal hypertrophy is itself the consequence of a chain of preceding events, it is obvious that its ultimate cause must be sought very soon after the operation, probably within the first 12 hours. Clearly before the stimulus to the growth can be understood, the growth itself must be examined in more detail than has previously been achieved. The earliest change previously detected by methods of chemical estimation was an increase in RNA content per cell, detectable by 12 hours post-operatively (Section 3.2.3.). Clearly changes in the surviving kidney must be occurring before this time. Since an increase in the content of RNA was the earliest detectable change with the methods so far used, it seemed reasonable to investigate RNA synthesis with more sensitive techniques. Indeed, as shown in section 3.5., measuring incorporation of a labelled

precursor, changes in RNA biosynthesis could be detected within a few hours of the operation.

Theoretically, however, the significance of this finding is uncertain. No change in the pattern of labelling of RNA fractions could be detected on sucrose density gradients. The means of detecting RNA biosynthesis in the remaining kidney - by comparing the ratio of the specific activity of kidney RNA to that of liver RNA with the corresponding ratio for control animals - is rather indirect, and it is conceivable that it does not give a true picture. For example, Peters (1963) has shown that, following unilateral nephrectomy, there is an increase in the excretion of water and of sodium ions by the remaining kidney during the first hour after the operation. It is possible, therefore, that there may be an increased flow of blood through the remaining kidney during the first hour. If this were the case, then more labelled precursor might enter the precursor pool and would be available for incorporation into RNA than would be the case in unoperated control animals. Thus the finding of a specific activity ratio higher in operated than in unoperated animals following injection of a labelled precursor might be explained, not as an increased rate of RNA biosynthesis in the remaining kidney, but as an increased availability of labelled precursor.

Malt and Stoddard (1966), however, have recently shown also that ribosomal RNA biosynthesis in the remaining kidney increases in the first few hours after unilateral nephrectomy in mice. Malt, Stoddard,

Miller and Keyes (1966) have in fact shown, using the sucrose density gradient technique, that following injection of [^{3}H]uridine in unilaterally nephrectomized mice, the 18s ribosomal RNA of the surviving kidney is labelled within 10 minutes of the operation. These workers reported that label does not appear in the 28s RNA until later and at 4 hours, all label was coincident with the extinction peaks. This last finding is in agreement with the results of the present investigation (Figure 33) but these workers have found a difference in the pattern of labelling of RNA of the remaining kidney which the present investigation failed to reveal. Although the techniques used were similar, there was a difference in the two investigations: whereas the present experiments were all performed on whole cell RNA, Malt et al. (1966) fractionated the cells and extracted and analysed only the RNA from the ribosomes. These workers must therefore have obtained clearer pictures than in the present studies for, as Figure 36 shows, with an incorporation time of 20 minutes, the labelling of whole cell RNA was very heterogeneous. (The patterns obtained are similar to those obtained by Kidson et al. (1963) with RNA from rat liver, by Hiatt (1962) and by Di Girolamo et al. (1966) with nuclear RNA from rat liver, by Hymer and Kuff (1964) with nuclear and with whole cell RNA of a plasma cell tumour and by Krantz and Goldwasser (1965) with RNA from bone marrow, all using very short incorporation times.) It is quite likely, however, that more meaningful results might have been obtained in the present

instance by fractionating the cells and examining RNA biosynthesis in nuclei and cytoplasm separately.

Significant increases in protein content per cell in the remaining kidney have been detected 24 hours after the operation (Table 35). Early changes in protein biosynthesis have not, however, been sought in the present investigation. Recently, Malt (1966) has studied polysome patterns in the kidney remaining after unilateral nephrectomy. Synthesis of protein is known to be carried out by ribosomal particles attached to messenger RNA (Brenner, Jacob and Meselson, 1961; Wettstein, Stahelin and Noll, 1963). This combination results in the formation of polysomes (Noll, Stahelin and Wettstein, 1963; Warner, Knopf and Rich, 1963) and the degree or rate of protein synthesis is related to the number of polysomes present and therefore to the quantity of messenger RNA available. Malt (1966) has shown that the rate of synthesis of polysomes was fastest 1 day after nephrectomy, dropped slightly in the second day, rose again to 7 days, after which it declined to normal by the fourteenth day.

Thus more information is now being gathered about the changes occurring in the surviving kidney at early periods after unilateral nephrectomy. It seems possible that an even more detailed study of the substances which accumulate immediately post-operatively might be of great value. They may provide the substrates which may be capable of inducing the formation or activation of enzymes, resulting in a synthesis of nucleic acids and proteins which have so far formed

the earliest means of detecting the process of compensatory renal hypertrophy.

S U M M A R Y

S U M M A R Y.

1. The estimation of kidney hypertrophy had been placed on a more quantitative basis by using the DNA content of the kidney as a measure of the cell number; an indication of the average cell composition has then been obtained by relating the other cellular components to DNA. This approach has been used to compare the hypertrophy following unilateral nephrectomy with the variation in kidney size and composition produced by variations in the diet. In addition, changes in RNA biosynthesis of the remaining kidney have been examined in the first 12 hours after unilateral nephrectomy.

2. In normal rats the right kidney is, on the average, about 7% heavier than the left and contains about 7% more RNA and DNA. The two kidneys have almost identical RNA and protein contents per cell.

3. There is a very good correlation between the weights of the right and left kidneys of normal rats. There is also a good correlation between right kidney weight and body weight. The correlation between total renal weight and body weight is slightly better than either that between right kidney weight and body weight or that between total renal weight and liver weight.

4. There is good correlation between the total contents of DNA, RNA and protein of the right kidney and the body weight of the animal. There is no correlation between the DNA concentration, RNA content per cell and protein content per cell on the one hand and

body weight on the other.

5. After unilateral nephrectomy the surviving kidney increases steadily in wet and dry weight, in rats and in mice, for the first 4 days after the operation. This is accompanied by a much slower increase in cell number, by a small increase in protein content per cell and by a dramatic increase in RNA content per cell. These changes are accompanied by an increase in the activity of deoxyribonuclease I and II. There is no consistent change in the activity of DNA deoxynucleotidyltransferase activity after unilateral nephrectomy and no detectable change in serum sodium, potassium or chloride or in blood haematocrit.

6. Changes in kidney weight and in total contents of DNA, RNA, protein and lipid phosphorus can also be produced by varying the protein content of the diet. These effects were found to be of approximately the same magnitude as the effects of unilateral nephrectomy on the remaining kidney 4 days post-operatively. Moreover the two effects seem to be independent of each other and approximately additive.

7. Starvation does not significantly affect the mean cell mass or content of RNA per cell of the surviving kidney in the first 36 hours after unilateral nephrectomy, but it does apparently abolish the increase in protein content per cell.

8. The feeding of a diet containing 10% of urea by weight for 4 days increases the mean cell mass, RNA content per cell and

protein content per cell of the kidneys of normal intact rats. Although these changes are similar to the effects produced in the remaining kidney after unilateral nephrectomy, they are only about one third as great.

9. The feeding of a diet containing 3% by weight of ammonium chloride for 6 days increases kidney weight by 25%, mean cell mass by 18%, RNA per cell by 19% and protein per cell by 18%. Equivalent amounts of ammonium citrate or sodium chloride do not produce these effects. The action of ammonium chloride would seem to be related to its acidotic effect rather than to the contribution it makes to either nitrogen excretion or electrolyte balance.

10. Decapsulation of the left kidney at the time of right unilateral nephrectomy does not affect the changes in weight or composition of the remaining kidney.

11. No consistent results could be obtained on RNA biosynthesis in the remaining kidney after unilateral nephrectomy using $[^3H]$ adenine as a precursor. The incorporation of $[^3H]$ orotic acid into the RNA of the surviving kidney was from 100 to 300 fold greater than the incorporation of $[^3H]$ adenine 4 hours post-operatively. Unilateral nephrectomy did not produce any qualitative changes in the pattern of incorporation of $[^3H]$ orotic acid into kidney RNA as demonstrated by the sucrose density gradient technique. Quantitatively, however, the ratio of the specific activity of kidney RNA to that of liver RNA was 25% to 120% greater in unilaterally

nephrectomized than in control animals at all times studied.

12. The RNA content per cell of the liver is increased substantially 2 days after removal of a 10% biopsy but is not affected by unilateral nephrectomy.

B I B L I O G R A P H Y

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