DIET AND TISSUE GROWTH.

IV. THE RATE OF COMPENSATORY RENAL ENLARGEMENT AFTER UNILATERAL NEPHRECTOMY IN THE WHITE RAT.*

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(Received for publication, October 7, 1926.)

The enlargement of the surviving kidney after unilateral nephrectomy is a well known and generally accepted fact. On the other hand, the character of this enlargement has been long disputed and has been the subject of many investigations. The literature on this point has been summarized by Arataki (1926, *b*) who, in his own studies on rats, found an increase in the size of both the glomeruli and the tubular systems, an hypertrophy, together with an actual cellular increase of the supporting tissue, an hyperplasia. He also believes that there is an hyperplasia of the constituent cells of the glomeruli and tubules.

On equally important phases of this subject, namely the rate of growth and the dietary factors concerned, there is a striking paucity of data. It is the purpose of this communication to report experimental studies on these questions.

As a result of the metabolic processes the nutritive fluid surrounding the tissues is vastly different both physically and chemically from the food ingested. The composition of this fluid medium of the cells changes comparatively little from day to day in a given species. It has been found possible experimentally to vary the quality of the food ingested within surprisingly wide limits without interfering with the normal functions of the organism. On the other hand, investigations on the effects of extreme or abnormal rations

* A preliminary report of a portion of this work was presented in the *Proceedings of the Society for Experimental Biology and Medicine* (Moise, T. S., and Smith, A. H., *Proc. Soc. Exp. Biol. and Med.*, 1925–26, xxiii, 561).

have disclosed the fact that there are certain unique dietary demands made by the body if growth and optimal functional activity are to be maintained. Insufficient energy intake, lack of essential amino acids, deficiency in certain inorganic salts and absence of accessory food factors all result in the disturbance of activity of certain organs and, grossly, in inhibition of growth. Recent studies have indicated, however, that when stunting occurs certain of the organ systems show a definite persistence of growth (Winters, Smith and Mendel). Smith and Moise (1924) have shown that injured liver tissue in rats is repaired on diets which are inadequate for growth of the body as a whole. It appears that certain organs are more sensitive to dietary insufficiency than others. The relation of diet to tissue growth is thus an intricate one and its investigation merits extension to other organs and systems.

Nothnagel (1886) early came to the conclusion that compensatory renal enlargement is a function of the composition of the blood. That this phenomenon is caused by some stimulus to function was later shown by Sacerdotti (1896) who, after performing unilateral nephrectomy, observed no increase in size of the remaining kidney when the animal fasted. However, when blood from a dog with total nephrectomy was injected into a normal animal without increasing the blood volume, there was evidence of renal enlargement. Enlarged kidneys have been observed in young rats grown on high protein foods and in mature rats given such diets, when both kidneys were present (Osborne, Mendel, Park and Darrow (1923), Reader and Drummond (1925), Jackson and Riggs (1926)). It appears from these experiments that in intact animals the level of non-protein nitrogen of the blood conditioned either by retention, on the one hand, or by abnormally high level of protein in the ration, on the other, influences directly the enlargement of the kidneys. That the same factor plays a large part in causing compensatory enlargement is shown in the present study.

The rate at which the total amount of renal tissue increases in response to these rather specific changes in blood composition is not only of academic interest but also of practical value. Osborne, Mendel, Park and Winternitz (1925) reported enlargement of the two kidneys in rats after they had been given protein-rich food for 1 week. It is of considerable importance in renal surgery to obtain similar statistics concerning the remaining kidney after nephrectomy. So far as we know such information on experimental animals is not available in the literature. Hinman (1923) gives data for the compensatory "hypertrophy" of the right kidney in rats after ligation of the left ureter without removing the kidney. In view of the scarcity of the published data, it seemed desirable to institute an extensive series of experiments on the problem of compensatory renal enlargement after unilateral nephrectomy.

It is obvious that any practical value of such results does not lie in the importance of the size of the organ *per se* but only in its function as correlated with its size. It has been pointed out by Oliver (1924) that in nephrectomized rabbits the weight of the kidneys is not a true index of the functional activity of the organ because the convoluted tubules and the glomeruli grow at unequal rates and urea is largely excreted in the tubules (Oliver, 1921). Studies of renal function to supplement the present investigation are in progress.

EXPERIMENTAL PART.

Methods.—The plan of the experiment was to remove the right kidney, place the animal immediately on the ration to be used and after a definite time interval to remove the remaining kidney for study. This procedure was carried out with the "standard" food, a diet providing the minimal amount of protein for optimal growth and maintenance and with the "high protein" food, which was unusual in that it contained a high concentration of protein. A third phase involved the study of the degree of compensatory renal enlargement after a constant time but with the animals eating diets containing increasing amounts of protein.

Adult white rats were used to eliminate the growth factor in renal enlargement, since it is well known that the organs increase in size as the body grows. Although a fluctuation in body weight occurred during the periods following nephrectomy, the authors believe that the change in size of the kidneys here recorded is one of practically uncomplicated compensation for the loss of kidney tissue as the result of the initial nephrectomy with the protein content of the diet as the only variable factor. An effort was made to use only male rats but in the early parts of the study non-pregnant females also were employed. None of the animals used had been given other than "stock" diets prior to being used in the present work. Individual variation, the chief difficulty inherent in animal experimentation, must be borne in mind in interpreting the present data. This variation may arise from differences either in heredity or in previous environment, which, in turn, might affect not only the gross reaction of the animal to the diet (efficiency of digestion and absorption) but also the response of the kidney cells to changes in blood composition. An effort was made therefore to use a relatively large number of rats for each experimental test period in order to eliminate as far as possible the uncertain factor of individual variation. It was planned to have twelve animals for each period in the study. In a few cases, accidental death reduced the number while in most instances more than twelve survived. In all, the data on considerably more than 300 rats were used in the final calculations.

In all the experiments diets composed of mixtures of purified foodstuffs were used. Casein furnished the protein, lard provided the fat, raw corn starch was used as a source of carbohydrate and an artificial salt mixture supplied the inorganic requirements. Cod liver oil mixed with the other ingredients and dried yeast fed apart from the food were used as sources of vitamines. In all the experiments the food and fresh tap water were given in unlimited quantity. It is important to insure an abundant supply of water, for with the protein-rich diets the large quantities of urea resulting from the metabolism of the protein constitute a potent diuretic and the increased volume of urine necessitates augmented fluid intake. In Table I is given the percentage composition of the various diets employed together with the approximate portion of the energy provided by the constituent foodstuffs. In planning the rations the aim has been to vary the proportion of protein while the other essential parts of the diet, *i.e.*, the salts and vitamines, remained unchanged. The caloric value of the diets containing 60 per cent or less of casein is approximately 5.3 Calories per gm. food. As the casein

TABLE I.

Composition of Experimental Diets.

	"Standard" food		Diet 30		Diet 45		Diet 60		Diet 75		"High protein" food		Diet 90	
	Part of diet	Part of total calories												
	per cent	*per cent*												
Protein (casein)[1]	18	14	30	23	45	35	60	46	75	60	85	76	90	85
Carbohydrate (raw cornstarch)	51	39	39	30	24	18	9	7						
Fat: lard	22	47	22	47	22	47	22	47	17	40	7	24		
Fat: cod liver oil	5		5		5		5		5		4		7	15
Salt mixture[2]	4		4				4		3		4		3	

300 mg. dried yeast daily given with each of the diets.

[1] A commercial product containing 13 per cent nitrogen.

[2] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1919, xxxvii, 557.

is increased above 60 per cent the energy value per gm. food becomes progressively less.

The method of caging used is the same as that heretofore described (Smith, Cowgill and Croll, 1925).

The technique of the operation was as follows: The rat was anesthetized with ether, the hair on the right flank was clipped closely and the area was painted with tincture of iodine. Under aseptic precautions an incision was made just below and parallel to the right costal margin. The peritoneum was incised and the kidney was lifted up by passing a small aneurysm needle through the pelvis. The perirenal fat was quickly dissected away, the pedicle ligated *en masse* and the kidney removed.

The wound was sutured in two layers and a thin collodion dressing applied. In the beginning of the experiments there was a small operative mortality from hemorrhage but this was eliminated by more complete dissection of the pedicle and thereafter there were no operative deaths. Wound infection was a negligible factor.

Early in the study the weight of the kidneys was obtained as well as linear measurements of the length, width and thickness. This method was finally discarded as unreliable on account of variations in the measurements of the small soft vascular viscus with slight pressure or with a change in the position of the organ to be measured. Immediately following its removal, the kidney, free of fat and hilic structures, was weighed in a stoppered bottle on an analytical balance. The manipulation was so standardized that the differences in amount of blood lost at various times is believed to have been negligible. After weighing, some of the kidneys were prepared for histological study and others were used for determinations of total solids.

The degree of enlargement was calculated as follows: On account of the change in the body weight of the rats during the period of study, the original (right) kidney weight was compared to that of one of Donaldson's (1924) rats of the same body weight and a factor obtained. The weight (A) of the enlarged kidney of the experimental rat was then multiplied by this factor to enable a direct comparison with Donaldson's figure to be made. From this product was subtracted the weight (B) of one kidney of a Donaldson rat weighing the same as the experimental rat at the end of the period. The difference is the actual enlargement and this divided by the weight (B) gives the enlargement in percentage. In view of the loss in body weight immediately following nephrectomy this method of calculation probably magnifies the degree of enlargement in the early periods, especially on the high protein diets. An example of the method of calculation is given below.[1]

Many measurements of both kidneys in normal rats have shown that they are either of the same weight or that the right is slightly heavier than the left. These observations are confirmed by Arataki (1926, *a*) who states that "on the average between birth and 350 days

	Initial body weight	Final body weight	Original kidney weight	Final kidney weight
	gm.	*gm.*	*gm.*	*gm.*
[1] Average of 12 rats present experiment	287	277	1.126	2.419
Donaldson's corresponding values	286.5	277.1	1.175	1.140

$$\text{Correction factor} = \frac{1.175}{1.126} = 1.04$$

$$\text{Corrected final kidney weight} = 2.419 \times 1.04 = 2.515 \text{ gm.}$$

$$\text{Difference (enlargement)} = 2.515 - 1.140 = 1.375 \text{ gm.}$$

$$\text{Degree of enlargement} = \frac{1.375}{1.140} \times 100 = 121 \text{ per cent}$$

the right kidney is 2.1 per cent heavier than the left in the male and 2.3 per cent heavier in the female. These differences are slight, but since the right kidney is also more often the heavier, they would appear to represent a real, though small, inequality." This fact introduces an error in some of the experiments in that the calculated percentages of enlargement are too low. However, these differences are so slight in view of the inevitable experimental difficulties involved in such work that they may be disregarded.

TABLE II.

Degree of Gross Enlargement of Remaining Kidneys of Rats Given "Standard" Food.

Interval after nephrectomy	Number of animals	Mean degree of enlargement
days		*per cent*
3	11	5
9	10	14
14	15	16
21	12	24
28	15	17
42	12	28
56	15	23
90	11	35
120	13	44
150	11	48

Results.

Changes in Body Weight.—After nephrectomy there was invariably a loss in weight of the experimental animals. The weight remained below the preoperative level in the group on "standard" food for a period of 2 to 4 weeks, by which time it had returned to the previous value and in many cases increased somewhat as the experiment progressed.

In the group on the "high protein" food the body weight decreased within 3 to 4 weeks following nephrectomy to a new level, often 30 or 40 gm. below the preoperative value, at which it remained throughout the experiment or only slowly rose after a period of 4 or 5 months toward the preoperative level. Concomitant with the initial loss in weight there was observed a decreased consumption of food. As the body weight of this group of rats became stabilized at this new level, the food intake rose until these rats were eating more gm. of food than those on the "standard" diet but, due to the lower caloric value of the "high protein" food, were actually ingesting less energy (see Smith and Carey, 1923). It appears, therefore, that after the immediate shock of the operation, the rise in nitrogenous waste products in the blood acts as a systemic depressant which is

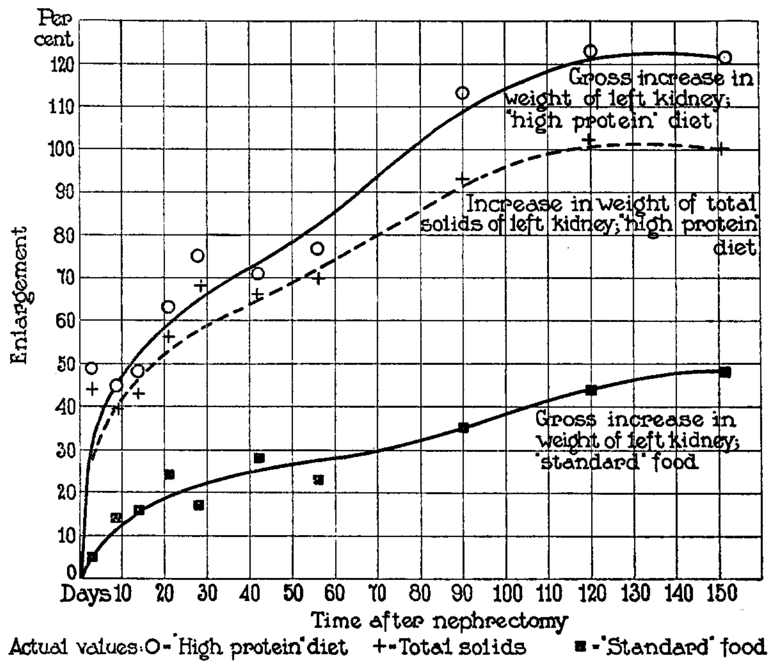

CHART 1. Rate of compensatory enlargement of remaining kidney after unilateral nephrectomy.

reflected in the appetite and body weight of the animal. These observations indicate that in mature rats, physiological well being may be adversely influenced not only by the well known method of decreasing the food intake but also by interfering with the excretion of substances which are primarily waste products. It is more than likely that a definite demonstration of this thesis could be produced in growing rats on protein-rich dietaries.

Rate of Kidney Enlargement.—The results of the study on the rats fed the "standard" food are summarized in Table II and in Chart 1. The individual values for the 3 day period were exceedingly variable, in some of the cases a decrease in size being observed. The recorded value of 5 per cent enlargement after 3 days is therefore only approximately correct. As may be seen from the data there is a rapid increase in the compensatory enlargement of the remaining kidney within the first 3 weeks following unilateral nephrectomy. The left kidney is about one-fifth heavier than the control at this time. The increase after 21 days and until the 120 day period appears to be progressive but at a definitely slower rate (approximately 3 per cent

TABLE III.

Degree of Gross Compensatory Enlargement after 21 Days on Diets of Varying Protein Content.

Casein in diet	Number of animals	Mean degree of enlargement
per cent		*per cent*
18	12	24
30	13	37
45	13	31
60	13	43
75	13	56
85	15	63
90	7	77

in 10 days). The data show that no significant enlargement takes place between the 120th and the 150th days. Hinman (1923) reported that in rats with one ureter ligated, the remaining kidney had reached its maximum compensatory enlargement on the average after 20 to 30 days which does not agree with what is here reported for nephrectomized rats. On the other hand, the data of Addis, Myers and Oliver (1924), when calculated in a way similar to the present method, show that at 106 to 125 days after nephrectomy the remaining kidney of the rabbit shows approximately 32 per cent enlargement, somewhat less than the value indicated for rats in the present study. The details of the diets in either of the above papers are not mentioned.

That the sudden response observed in the early periods is a reaction to the blood composition and the consequent increased demand for renal activity seems probable. Although Bürger and Grauhan (1923) have shown that after nephrectomy in the human there is a pronounced increase in nitrogen excretion, the very important part played by the nature of the diet is shown subsequently in the present study.

The second phase of the experiment demonstrates that dietary protein is at least one of the factors conditioning the compensatory enlargement of the kidney. The plan was altered somewhat for these tests. It was observed in the preceding section that on the

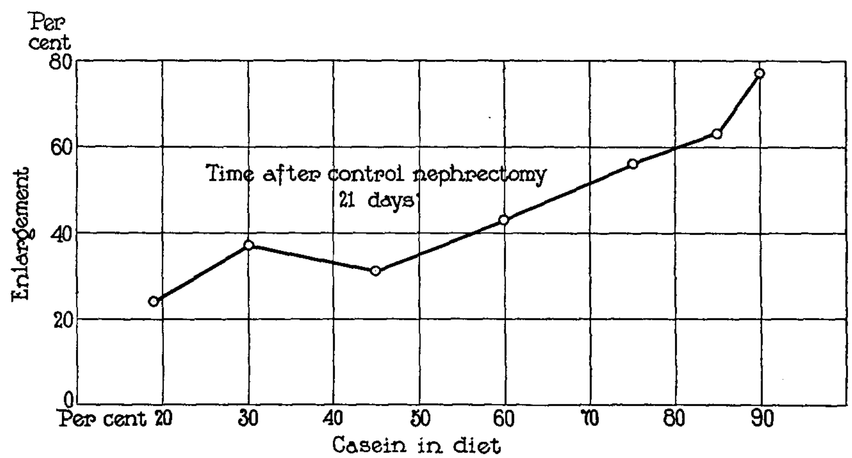

CHART 2. Relation of renal compensatory enlargement to the amount of dietary protein.

"standard" food a marked degree of enlargement could be demonstrated in 21 days. In this second phase the time interval after the first nephrectomy was maintained constant at 21 days and diets with increasing concentrations of protein were used with different groups of rats. Table I shows the rations used.

In Table III are given the data for Chart 2 which shows the degree of enlargement plotted against the per cent of casein in the food. No attempt has been made to draw a "smoothed" curve but, as shown, it is plain that there is almost a direct proportion between the degree of compensatory enlargement and the concentration of dietary protein. In these experiments the catabolic non-protein nitrogen of Bürger and Grauhan (1923) is constant, since the animals were all treated in the same way and the measurements were made at the same time. Moreover, their published protocols show that in humans the catabolic nitrogen is eliminated within a week. It is obvious, therefore, that the enlargement illustrated by the present data is brought about by some condition depending primarily on the composition of the diet, more specifically, the protein concentration.

What is the maximum compensatory enlargement to which the remaining kidney may be forced under stress resulting from excess dietary protein? To answer this question a series of experiments was planned identical with the first series except that the "high protein" food was used (see Table I). This ration, though extreme, contained all the recognized dietary essentials for growth and maintenance. The data obtained are summarized in Table IV and expressed graphically in Chart 1. The curve showing the rate of enlargement is similar in shape to that of the first series with "standard" food except that the values are much greater. There is the analogous rapid increase in size within the early intervals followed by a slower increase to a maximum. However, where, in the first group on "standard" food, the enlargement at 30 days was about 20 per cent, on the "high protein" diet in a similar length of time the remaining kidney weighed 70 per cent more than the control. The increase progressed at the rate of about 7 per cent in 10 days to the 90th day when the compensatory enlargement was about 113 per cent. From the 90th to the 120th day the rise is much slower and from the 120th day to the 150th day there appears to be no significant increase, the value at 150 days being 121 per cent. It seems probable from these data that even under the stimulus of such a protein-rich diet as here used the maximum enlargement is reached little sooner than on the "standard" food with a relatively low protein content. These combined data show the tremendous physiological reserve possessed by the rat kidney and provide striking evidence for Meltzer's (1906) "factor of safety" in the organism.

The results suggest several questions. Does the increased kidney weight observed after compensatory enlargement result from a *bona fide* increase in renal tissue or is it due to an accumulation of fluid in the kidney? Many comparisons have been made between the total solids of normal kidneys and of those showing the extreme enlargement on the protein-rich diet. Table V summarizes the data bearing on this point. The average percentage of total solids in the enlarged kidneys in the longer periods, *i.e.*, from 90 to 150 days, is lower than that of normal kidney tissue and the difference is a real one. The total solids per cent of the enlarged kidneys of the 21 to 56 day group is larger but there still is a small though significant variation from the normal. The values for the 3 to 14 day group approaches that of the normal slightly more closely than those of the other two

TABLE IV.

Degree of Compensatory Enlargement of Kidneys of Rats Given "High Protein" Food.

Interval after nephrectomy	Number of animals	Mean degree of enlargement	Mean increase of total solids
days		*per cent*	*per cent*
3	12	49	44
9	13	45	40
14	13	48	43
21	15	63	56
28	14	75	68
42	15	71	64
56	14	77	70
90	10	113	93
120	12	123	102
150	12	121	100

groups. With these data the net tissue enlargement has been calculated for the group on "high protein" food and the results are shown in Chart 1. The degrees of compensatory renal enlargement discussed heretofore in this paper are the gross increase in size, *i.e.*, including the water, the basis upon which other reports in the literature have been made. It is thus obvious that the renal enlargement resulting from extreme conditions of stimulation to functional activity, while a real one, is accompanied by a definite increase in the water content of the kidney and that this is proportional to the time interval during which the animal is given the experimental diet.

Arataki (1926, *b*) has shown that the compensatory enlargement of the remaining kidney is due mainly to the hypertrophy of the glomeruli and tubules. It is possible that the increase in the fluid content of the enlarged kidneys observed in the present experiments is due to its being mechanically retained in the enlarged tubules.

TABLE V.

Total Solids of Kidneys.

Group	Number of rats in groups taken	Degree of enlargement	Mean total solids	Probable error	Significant difference from normal* $\frac{D}{PEd}$
		per cent	*per cent*	*per cent*	
Normal..................	25		22.3 ± 0.78	±0.101	
"*High protein*" *food***					
90, 120 and 150 day periods...............	15	113–123	20.2 ± 0.45	±0.078	16.1 Positive
21, 28, 42 and 56 day periods...............	19	64–77	21.4 ± 0.92	±0.15	5.0 Positive
3, 9 and 14 day periods...	14	45–49	21.5 ± 0.56	±0.102	5.7 Positive

* D = actual difference between the two averages being compared.

PEd = probable error of the difference.

Difference between the two means is significant when $\frac{D}{PEd} > 3$. (See Sherman, H. C., Chemistry of food and nutrition, New York, 3rd edition, 1926, 604.)

** The periods were grouped in this manner in order to obtain greater accuracy by using larger numbers of cases. As observed from the chart there is little or no significant difference between the values for gross enlargement within the groups.

The weights of the heart and liver of some of the rats showing the greatest degree of renal enlargement on the "high protein" diet were compared with similar data for normal animals taken from Donaldson (1924). In no case was an increase in size of either of these organs demonstrable. This observation agrees with that reported by Osborne, Mendel, Park and Darrow (1923), and with many unpublished observations of these investigators.[2]

[2] Personal communication of Dr. Mendel.

Has the renal function after nephrectomy returned to normal before the final structural equilibrium has been reached or even when this stage is reached? Data bearing on this question have been published by Addis, Myers and Oliver (1924) who used nephrectomized rabbits as the experimental animals. They found that after 106 to 125 days when the *total* renal tissue was 66 per cent of that before nephrectomy the function as measured by the rate of urea excretion was 98 per cent of the normal. The values for rate of compensatory enlargement used by them were those of Hinman (1923) for rats. It seemed necessary to begin new experiments on renal function of rats under the conditions of the present study and the work is now in progress.

Finally, do these extremely large kidneys show evidences of tissue injury? The effect of diets rich in protein on the kidney is at present a favorite theme for debate. The material obtained from the present investigation should afford unique opportunity for such a study, the results of which will soon be published.

Complete protocols of all the experiments have been filed in the archives of the Wistar Institute, where they may be consulted.

SUMMARY.

The rate of compensatory enlargement of the remaining kidney after unilateral nephrectomy has been studied in adult rats fed diets containing various concentrations of protein.

A curve of enlargement on "standard" food (18 per cent casein) shows a rapid initial increase with subsequent slower rise to the 120th day. There is no significant difference between the value at 120 days (44 per cent) and that at 150 days (48 per cent).

A similar series with diets containing increasing concentrations of protein but with a constant time interval (21 days) after nephrectomy shows an increase in the degree of enlargement directly proportional to the protein content of the food. The values vary from 24 per cent with the 18 per cent casein ration to 77 per cent with the 90 per cent casein diet.

A third series shows the enlargement on "high protein" food (85 per cent casein). The values vary from 49 per cent at 3 days to 121

per cent at 150 days. There is no significant difference between the value at 120 days (123 per cent) and that at 150 days (121 per cent).

Determinations of total solids on the experimental kidneys show that the recorded enlargement involves mainly an actual tissue increase.

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