CLXXXII. AN INVESTIGATION OF THE CAUSE OF RENAL HYPERTROPHY IN RATS FED ON A HIGH PROTEIN DIET.

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The problem of the enlargement of the kidneys of rats fed on diets rich in protein has, in recent years, been receiving considerable attention notably by Addis and the Mackays [1926, 1, 2] who have shown that the hypertrophy is proportional to the protein consumption and have elaborated a formula which expresses this relationship in terms of protein consumed and kidney weight per unit of surface area. The cause of this enlargement, however, still remains obscure. The excretion of urea naturally suggests itself as a causal factor. The feeding of urea, however, in amounts equivalent to what would be excreted on a high protein diet has been shown to produce a much smaller effect on the kidneys [Osborne *et al.*, 1926; Mackay, Mackay and Addis, 1931]. The effect of feeding proteins of different origin has however scarcely been investigated. Osborne *et al.* [1926] found that caseinogen, gliadin and beef residues all produced an enlargement of the kidneys but no actual numerical data on the basis worked out by the Mackays and Addis [1927; 1928] were elaborated. The object of the following experiments was an attempt to find out the cause of this hypertrophy.

Two possibilities suggest themselves. Do the kidneys in the rat form a depot for reserve protein, or is the hypertrophy a response to an increase in physiological function? It is possible in this connection that the kidneys are associated in some way with the intermediary metabolism of protein quite apart from the mere excretion of nitrogenous end-products.

Experimental.

A series of experiments was carried out to compare the following proteins and protein derivatives, and in addition the effect of an acid and alkaline diet—caseinogen, liver, gelatin, wheat gluten, glycine, glutamic acid, urea, NaH_2PO_4 and $NaHCO_3$. The experimental procedure adopted in these experiments was practically the same as that employed by the Mackays [1927]. Standard albino rats 30 days old and obtained from the same source as far as each particular series of experiments is concerned were employed throughout. They were kept in cages open to the front with not more than 6 rats to a cage. The sexes were kept apart. The room in which they were kept was well lit and open to the south and was maintained fairly constant in temperature day and night. Water and food were allowed *ad lib.*, and the food consumption was measured each day. The rats were weighed about every third day. The duration of the experiments varied from 30 to 39 days in the different series. At the end of the experimental

period the rats were killed by chloroform and weighed. The abdomen was then opened, the kidneys removed, decapsulated, split in half, dried on filter-paper and weighed in a glass-stoppered bottle. The two kidneys were weighed together and the average taken as the weight of one. The surface area was calculated by Meeh's formula $S = BW^{\frac{2}{3}} \times K$. The constant $K = 11{\cdot}36$ was that employed by the Mackays [1927] and found to be applicable to the rat by Carman and Mitchell [1926]. From the experimental data the following calculations have been made for each series. The average increase in body weight per rat daily; the average kidney weight per 100 cm.^{2} body surface. The average daily protein consumption per 100 cm.^{2}; the average increase in body weight per g. of protein consumed.

The first series of experiments was carried out with liver, gelatin and caseinogen. The object of testing liver was partly because it offers a protein differing considerably from caseinogen and partly because it is rich in extractives and nucleoproteins. Gelatin was chosen as a test protein because it has a low biological value and hence might be considered inadequate to build up any reserve tissue in the kidney. A further point of note is that gelatin is poor in tryptophan, cystine and tyrosine and according to certain workers [Newburgh and Marsh, 1925] these amino-acids are a contributing cause of the renal degeneration found in rats fed over a long period on a high protein diet. The diets employed are given in Table I and the results in Table II.

Table I. *Diets employed.*

(Figures represent parts per 100.)

Series	Dried liver	Casei-nogen	Gela-tin	Glu-ten	Gly-cine	Glu-tamic acid	Dex-trin	Lard	Urea	Salt mix-ture	Cod-liver oil	Dried yeast	Agar	$NaHCO_3$	NaH_2PO_4	Total protein %	Total Cals.
A	80	—	—	—	—	—	—	—	—	3	6	9	2	—	—	50·5	434
B	—	16	37	—	—	—	15	8	—	3	10	9	2	—	—	50·6	436
C	—	57	—	—	—	—	9	10	—	3	10	9	2	—	—	50·0	436
D	—	20	32	—	—	—	13	5	—	3	10	9	2	—	6	48·8	396
E	—	20	32	—	—	—	13	5	—	3	10	9	2	6	—	48·8	396
F	—	57	—	—	—	—	8	5	—	3	10	9	2	—	6	48·8	402
G	—	20	—	—	—	—	51	5	—	3	10	9	2	—	—	20·25	428
H	—	20	32	—	—	—	17	7	—	3	10	9	2	—	—	48·9	431
I	—	57	—	—	—	—	10	10	—	3	10	9	2	—	—	48·9	429
J	—	20	—	—	—	—	51	5	—	3	10	9	2	—	—	20·6	428
K	—	57	—	—	—	—	9	10	—	3	10	9	2	—	—	48·5	429
L	—	20	—	—	—	—	34·2	12	9·8	3	10	9	2	—	—	48·8*	426
M	—	20	32	—	—	—	17	7	—	3	10	9	2	—	—	48·5	431
N	—	20	—	—	—	—	56	—	—	3	10	9	2	—	—	20·2	402
O	—	20	—	26	—	—	30	—	—	3	10	9	2	—	—	42·7	402
P	—	20	—	—	10	—	42	4	—	3	10	9	2	—	—	31·4	403
Q	—	20	—	—	—	25	27	4	—	3	10	9	2	—	—	34·1	402

* Urea-N calculated as protein.

It will be noted that there is no difference in the kidney weights of groups A and C, *i.e.* those fed with liver and caseinogen respectively. The rats on the gelatin diet (B) showed however a considerable hypertrophy in comparison with groups A and C in spite of the fact that they actually consumed less protein. In order to ascertain if the differences between the groups are significant the standard deviation σ was calculated for each group and the probable error of the difference of the means calculated by the formula $0{\cdot}6745\sqrt{\frac{\sigma_1}{n_1}+\frac{\sigma_2}{n_2}}$ where n_1 and n_2 are the number of observations in each group and σ_1 and σ_2 the corresponding standard deviations. Where the ratio of the difference of the means to the probable error of the difference of the means is 3 or more the result is considered significant. The following are the data for series I.

A	σ	B	σ	C	σ
Males	0·03507	Males	0·00319	Males	0·02097
Females	0·01552	Females	0·0900	Females	0·02097

A and B males:	Difference of means	0·066
	Probable error of difference of means	0·0113
	Ratio	5·84 : 1
A and B females:	Difference of means	0·103
	Probable error of difference of means	0·025
	Ratio	4·12 : 1
B and C males:	Difference of means	0·084
	Probable error of difference of means	0·0082
	Ratio	10·24 : 1
B and C females:	Difference of means	0·099
	Probable error of difference of means	0·025
	Ratio	3·96 : 1

It will be seen that the difference is significant when the group fed with gelatin is compared with either of the groups fed with caseinogen or liver. It is possible that the increase in kidney weight of group B (gelatin) is due to the

Table II.

			Av. increase in body weight per rat per day, g.	Av. kidney weight per 100 cm.² g.	Av. protein consumption per day per 100 cm.² g.	Av. increase in body weight per rat per g. protein consumed
Series 1 31 days exp.	A	7 ♂	3·07	0·285	1·64	0·57
		7 ♀	2·10	0·239	1·57	0·52
	B	9 ♂	2·13	0·351	1·33	0·59
		6 ♀	1·76	0·342	1·58	0·46
	C	5 ♂	3·70	0·267	1·38	0·75
		12 ♀	2·03	0·243	1·39	0·51
Series 2 32 days exp.	D	6 ♂	1·12	0·403	1·50	0·32
		6 ♀	0·96	0·363	1·33	0·30
	E	6 ♂	2·0	0·395	1·43	0·46
		6 ♀	1·09	0·338	1·27	0·33
	F	6 ♂	3·05	0·324	1·41	0·62
		6 ♀	2·02	0·348	1·33	0·53
Series 3 39 days exp.	G	5 ♂	2·96	0·265	0·65	1·14
		6 ♀	1·67	0·227	0·67	0·81
	H	6 ♂	1·95	0·362	1·39	0·47
		6 ♀	1·34	0·340	1·39	0·38
	I	6 ♂	2·90	0·265	1·28	0·63
		6 ♀	1·60	0·237	1·38	0·40
Series 4 32 days exp.	J	6 ♂	2·01	0·218	0·67	1·07
		6 ♀	1·39	0·196	0·70	0·80
	K	6 ♂	1·73	0·221	1·14	0·59
		6 ♀	1·62	0·246	1·52	0·45
	L	6 ♂	1·89	0·236	1·44	0·52
		6 ♀	1·55	0·217	1·44	0·43
	M	6 ♂	1·63	0·369	1·49	0·47
		6 ♀	1·20	0·336	1·69	0·32
Series 5 32 days exp.	N	5 ♂	1·91	0·212	0·65	1·26
		5 ♀	1·57	0·199	0·64	1·16
	O	5 ♂	1·79	0·304	1·31	0·59
		5 ♀	1·31	0·270	1·31	0·47
	P	3 ♂	0·65	0·278	1·10	0·59
	Q	4 ♀	1·17	0·244	1·12	0·52

fact that although this group consumed less protein than groups A and C they actually catabolised more nitrogen. This assumption might be expected *a priori* since gelatin is almost incapable of promoting growth. This explanation would assume however that the hypertrophy is observed only when the protein is actually catabolised. In point of fact, as is seen from Table II, the increase in body weight per g. of protein consumed is approximately the same in groups A and B. It cannot however be inferred that the material added to the body in each group had the same nitrogen content.

In series 2 an attempt was made to repeat the work of Addis, Mackay and Mackay [1926, 1, 2] on the influence of adding acid or alkali to the diet. Nash and Benedict [1921] have shown that the kidney, in the dog at least, is the main if not the only organ concerned in the formation of ammonia and it was thought possible that the kidney hypertrophy might be secondary to an increased activity of this function following a high protein diet. The salts employed in the experiments were sodium dihydrogen phosphate and sodium bicarbonate. In experiments D and E (Table I, series 2) the diets were identical except that D was acid and E alkaline. The additional protein in D and E was gelatin. Mackay, Mackay and Addis [1926] have shown that phosphates themselves cause a considerable increase in kidney weight, and hence in groups D and E it would be possible to see if the hypertrophy of the kidneys in group D with gelatin and acid phosphate would be greater than in group E with gelatin and alkali. In short would there be a summation of those two factors in bringing about a hypertrophy? The experimental data are given in Table II, series 2.

It will be noted that there is scarcely any difference between groups D and E fed with gelatin and acid and alkali respectively. The difference in the kidney weights for the males is only 0·008 g. and for the females 0·025 g. per 100 cm.^{2} body surface. Such differences are not significant. It thus appears that there is no summation of the effects of gelatin and phosphates when fed together in a diet. In group F fed with caseinogen *plus* acid phosphate the effect of the salt is quite marked. This is seen most clearly when groups C and F of series 1 and 2 are compared. The following are the statistical data.

D	σ	E	σ	F	σ
Males	0·03014	Males	0·0250	Males	0·0442
Females	0·07200	Females	0·0375	Females	0·0820

Series 2, D and F males:	Difference of means	0·079
	Probable error of difference of means	0·0147
	Ratio	5·37 : 1
Series 2, E and F males:	Difference of means	0·071
	Probable error of difference of means	0·139
	Ratio	5·07 : 1
Series 1 and 2, C and F males:	Difference of means	0·057
	Probable error of difference of means	0·01371
	Ratio	4·15 : 1
Series 1 and 2, C and F females:	Difference of means	0·105
	Probable error of difference of means	0·0214
	Ratio	4·90 : 1

Taking series 2 alone to begin with it will be noted that the difference between group D (gelatin and acid phosphate) and group F (caseinogen and acid phosphate) is significant in respect of the males. The kidney weights of the females of those two groups are however approximately the same. This contrast in the effect of caseinogen in series 2 as compared with series 1 must be attributed to the phosphates as the statistical data show when groups C and F of series 1 and 2

are compared above. The difference is significant for both sexes. The evidence from these experiments does not appear to favour the hypothesis that acidity or alkalinity of the diet, and hence ammonia formation by the kidney, is a factor in the renal hypertrophy. In series 3 an attempt was made to test the relative effect of superimposing caseinogen on the one hand and gelatin on the other on a diet low in caseinogen. The diets employed and the experimental data are given in Tables I and II, series 3 (G, H, I). Contrary to the findings of the Mackays [1927] there is no increase in the kidney weight when the protein of the diet is increased by the addition of extra caseinogen (groups G and I). In group H however where the increase in the protein of the diet is in the form of gelatin a hypertrophy of the kidneys is to be noted. The following are the statistical data.

G	σ	H	σ	I	σ
Males	0·03301	Males	0·02696	Males	0·02720
Females	0·03684	Females	0·03390	Females	0·02260

Series 3, G and H males:	Difference of means	0·097
	Probable error of difference of means	0·0124
	Ratio	7·82 : 1
G and H females:	Difference of means	0·113
	Probable error of difference of means	0·0137
	Ratio	8·25 : 1
H and I males:	Difference of means	0·097
	Probable error of difference of means	0·0101
	Ratio	9·63 : 1
H and I females:	Difference of means	0·103
	Probable error of difference of means	0·112
	Ratio	9·19 : 1
G and I males:	Difference of means	Nil
G and I females:	Difference of means	0·010

The increase in weight between groups G and H and H and I is very marked and merits special attention when comparing groups H and I which had practically the same percentage of protein in the diet and consumed almost the same quantity of protein per 100 cm.^{2} body surface. Owing to the fact that additional caseinogen failed to cause a hypertrophy in the above series it was decided to repeat the experiments and in addition to compare the effect of urea and gelatin respectively with each other and with caseinogen. The diets employed and the experimental data are given in Tables I and II, series 4. Below are recorded the statistical results (J, K, L, and M).

The results in general confirm those of series 3. The increase in kidney weight on the high protein diet (K) as compared with the low (J) is significant with the female but not with the male group. The increase in kidney weight with the group fed with urea (L) as compared with the low protein (caseinogen) group (J) is significant but the differences are much less than those between groups J and M where the extra nitrogen is consumed in the form of gelatin. The difference is very marked in the case of groups L and M which consumed approximately the same amount of nitrogen—group L largely as urea and group M as gelatin. Assuming that the gelatin was all catabolised and excreted as urea, this experiment would confirm the fact that the excretion of urea is not the causal factor in the kidney hypertrophy. From the experiments in series 3 and 4 an estimate can be made of the increase in kidney weight per 100 cm.^{2} body surface per

J	σ	K	σ	L	σ	M	σ
Males	0·0122	Males	0·0232	Males	0·0104	Males	0·0264
Females	0·0187	Females	0·0176	Females	0·0150	Females	0·0264

Series 4, J and M males:	Difference of means	0·151
	Probable error of difference of means	0·008
	Ratio	18·8 : 1
J and M females:	Difference of means	0·140
	Probable error of difference of means	0·008
	Ratio	17·5 : 1
J and K males:	Difference of means	0·003
	Probable error of difference of means	0·0072
	Ratio	0·041 : 1
J and K females:	Difference of means	0·050
	Probable error of difference of means	0·0067
	Ratio	7·64 : 1
J and L males:	Difference of means	0·018
	Probable error of difference of means	0·0044
	Ratio	4·09 : 1
J and L females:	Difference of means	0·021
	Probable error of difference of means	0·0062
	Ratio	3·38 : 1
L and M males:	Difference of means	0·133
	Probable error of difference of means	0·0078
	Ratio	17·02 : 1
L and M females:	Difference of means	0·119
	Probable error of difference of means	0·009
	Ratio	13·22 : 1

additional g. of protein consumed. In series 3 this has been calculated where the increase in protein consumed was derived from gelatin. The following are the values found.

Series 3, males	0·131 g. increase in kidney weight per 100 cm.^{2} per extra g. protein consumed per 100 cm.^{2}
females	0·156 g. increase in kidney weight per 100 cm.^{2} per extra g. protein consumed per 100 cm.^{2}
Series 4, males	0·184 g. increase in kidney weight per 100 cm.^{2} per extra g. protein consumed per 100 cm.^{2}
females	0·141 g. increase in kidney weight per 100 cm.^{2} per extra g. protein consumed per 100 cm.^{2}

These figures agree fairly closely with those obtained by the Mackays [1927] where the additional dietary protein was in the form of caseinogen.

In series 5 a cereal protein was tested and an attempt was made to find out which particular amino-acid or acids, if any, were responsible for the hypertrophy observed when feeding the whole protein. Wheat gluten, glycine and glutamic acid were selected as examples of cereal protein and amino-acids, glycine being chosen because gelatin contains a high percentage of this particular amino-acid and glutamic acid because of its high concentration in gluten. In the experiment with glycine 4 male rats were put on a diet containing 20 % of this substance; they tended to lose weight however and finally one died. The percentage of glycine was then reduced to 10 %. The animals in group Q however consumed a diet containing 26 % of glutamic acid quite readily. The diets and experimental data are given in Tables I and II (N, O, P and Q). It will be noted that both gluten and the amino-acids caused a hypertrophy of the kidneys. The following are the statistical data.

N	σ	O	σ	P	σ	Q	σ
Males	0·0275	Males	0·0356	Males	0·328		—
Females	0·0250	Females	0·0317		—	Females	0·0091

Series 5, N and O males:	Difference of means	0·094
	Probable error of difference of means	0·0135
	Ratio	6·96 : 1
N and O females:	Difference of means	0·070
	Probable error of difference of means	0·0122
	Ratio	5·37 : 1
N and P males:	Difference of means	0·066
	Probable error of difference of means	0·0152
	Ratio	4·34 : 1
N and Q females:	Difference of means	0·45
	Probable error of difference of means	0·0091
	Ratio	4·51 : 1

The differences in the weights of the kidneys per 100 cm.^{2} per additional g. of protein (calculated from nitrogen consumed) consumed per 100 cm.^{2} body surface have been calculated for the different groups, *e.g.* caseinogen-gluten where the additional protein refers to the last-mentioned.

N and O males (caseinogen-gluten) 0·139 g. increase in kidney weight per 100 cm.^{2} per extra g. protein consumed per 100 cm.^{2}

N and O females (caseinogen-gluten) 0·105 g. increase in kidney weight per 100 cm.^{2} per extra g. protein consumed per 100 cm.^{2}

N and P males (caseinogen-glycine) 0·146 g. increase in kidney weight per 100 cm.^{2} per extra g. protein consumed per 100 cm.^{2}

N and Q females (caseinogen-glutamic acid) 0·093 g. increase in kidney weight per 100 cm.^{2} per extra g. protein consumed per 100 cm.^{2}

O and P males ((caseinogen glycine)-(caseinogen gluten)) 0·123 g. increase in kidney weight per 100 cm.^{2} per extra g. protein consumed per 100 cm.^{2}

O and Q females ((caseinogen glutamic acid)-(caseinogen gluten)) 0·136 g. increase in kidney weight per 100 cm.^{2} per extra g. protein consumed per 100 cm.^{2}

If comparisons are drawn between animals of like sex it will be seen that the increase in kidney weight is proportional to the additional protein, *i.e.* N consumed. The addition of gluten to the diet causes an increase in the weight of the male kidneys of 0·139 g. per 100 cm.^{2} body surface per additional g. of protein consumed. Comparing the females (N and O) on caseinogen and caseinogen *plus* gluten respectively with those on caseinogen and caseinogen *plus* glutamic acid respectively (N and Q) the differences are 0·105 g. and 0·093 g. per unit of surface per additional g. of protein consumed. Taking into consideration the nature of the experiment these results are fairly close, a difference of only 0·012 g. being observed between the groups. A similar comparison between the males of groups O and P (gluten and glycine respectively) against N as a standard shows a difference of only 0·007 g. between the groups. These results indicate that it is unlikely that any particular amino-acid is the factor concerned in the hypertrophy. It should be pointed out however that these differences are smaller than those obtained when gelatin is fed. In series 3 the figures giving the increase in kidney weight with gelatin as the protein superimposed are males 0·131 g. and females 0·141 g. per unit of surface area per additional g. of protein consumed. The corresponding figures of series 4 are males 0·184 g. and females 0·141 g. The averages of the two series—males 0·157;

females 0·148 g.—however, are approximately the same as those obtained by the Mackays [1927] where caseinogen at different levels was the protein fed. It will be recalled that in our experiments additional caseinogen caused a relatively small hypertrophy of the kidneys and in some cases none at all.

At this stage of the investigation however it cannot be said what is the cause of the hypertrophy. That it is a real increase in tissue substance and not a hydraemia has been shown in a recent paper by Mackay [1933], who found that the moisture content for all kidneys fell between 74 and 76 %. In confirmation of his findings the average water contents of the kidneys in series 5 exhibit the same consistency as shown below.

	H_2O %
Group N, males	75·1
females	73·7
Group O, males	76·5
females	76·4
Group P, males	76·0
Group Q, females	74·9

From the general trend of evidence however in the recent paper by Mackay [1933], which has just come to our notice, and from the results given in this paper, it appears that the cause of the hypertrophy is associated with some change common to all the amino-acids. Ammonia formation and deamination naturally suggest themselves. The first possibility appears unlikely in view of the experiments recorded here and by Addis and the Mackays [1926, 1, 2]. The question as to whether the kidney is capable of deaminating merits special attention in view of the work of Bollmann, Mann and Magath [1926], who have shown that, as far as the dog is concerned, the liver is the main if not the only organ capable of deaminating. Recent work by Holmes and Watchorn [1927] shows that the growing embryonic rat kidney produces ammonia and urea *in vitro*. Krebs [1932] has further shown that rat kidney *in vitro* is more active than rat liver in deamination and ammonia formation. Such results suggest strongly that, at least in the rat, the kidney may be concerned in deamination. In support of this hypothesis is the fact that the hypertrophy tends to be larger or more readily produced by the addition of gelatin than of caseinogen to the diet. It can be more or less assumed that ingested gelatin will be almost entirely catabolised while some of the caseinogen may readily be used for anabolic purposes. Experiments on the actual nitrogen excretion on the two diets would give information on this point. The application of these results to the treatment of renal disease can hardly be ventured as yet. Some of the kidneys of each group of series 3 were examined histologically and no abnormality was found as had already been noted by Osborne *et al.* [1926]. Newburgh and Curtis, however [1928], find definite signs of degeneration in the kidneys of rats fed over long periods on diets containing a high percentage of protein.

Summary.

1. The increase in kidney weight of rats fed with a number of proteins and protein derivatives has been investigated.

2. Gelatin tends to produce a more marked increase in kidney weight than either caseinogen or liver.

3. Glycine, glutamic acid and gluten all produce an increase which is more or less proportional to the additional nitrogen consumed.

4. It is considered likely that the hypertrophy is associated with some stage in the intermediary metabolism of protein—probably deamination by the kidney.

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