XVI. THE ROLE OF THE LIVER IN HIPPURIC ACID SYNTHESIS.

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THE attempt will be made in the following pages to show that by the use of hydrazine, a specific agent affecting liver tissue and leaving that of the kidney intact [Wells, 1908; Underhill and Kleiner, 1908], it is possible to demonstrate a considerable participation of the liver in hippuric acid conjugation. The studies of Bunge and Schmiedeberg [1876], followed by the corroborating studies of Kochs [1879] and of Bashford and Cramer [1902], have been interpreted as decisive evidence of a difference in power of effecting hippuric acid pairing on the part of carnivores, including man, and herbivores, in that the seat of the process in herbivores is not limited to the kidney as in the case of the carnivores. This conclusion remained practically unchallenged until Kingsbury and Bell [1915, p. 301] found "a relatively large amount of hippuric acid in the liver of a nephrectomised dog after injection of glycocoll and sodium benzoate" suggesting "that the liver like that of the rabbit may possess this synthetic power."

In the present set of experiments we have departed from the procedure followed by other investigators in two respects, namely, that we have utilised the urine rather than the blood, and secondly, we have employed a reagent which may be used subcutaneously to cause liver involvement while leaving the kidney intact without introducing the additional factor of operation, ligaturing, etc.

METHODS.

Two male dogs, weighing respectively 9.05 and 9.50 kg., were isolated in metabolism cages. The urine was collected in 24 hour quantities, thymol in alcohol being used as a preservative; faeces were excluded by use of glass-wool and filter plates. The food consisted of dog-biscuits supplemented by fresh beef, chicken trimmings and boiled meat from the soup kitchen. The intake of water and of chloride was noted; likewise we noted carefully the general condition of the dogs especially after the administration of reagents. The dogs

were kept in the cages several days before the collection of urine was commenced in order that they might become accustomed to their surroundings and also that their controlled food might replace that previously given them.

In the administration of sodium benzoate (2 g.) and of hydrazine sulphate (500 mg.) by mouth, we wrapped the substances in fresh steak formed into a bolus which the dogs swallowed freely, thus insuring that the animals received the complete amount of the drug. Subcutaneous injections of 500 mg. hydrazine sulphate dissolved in 20 cc. of sterile water were given into the flanks under aseptic conditions.

Hippuric acid was determined in 50 cc. aliquots of urine after the method of Folin and Flanders [1912], the reduction in amount of urine from 100 cc. to 50 cc. being compensated for by reducing the amounts of the other reagents, nitric acid, ammonium sulphate, sodium chloride, chloroform, etc., correspondingly.

Chlorides were determined by the Volhard method, for renal efficiency. Amino-nitrogen was determined gasometrically upon the hydrolysed urine. Free benzoic acid was determined upon the residue from 50 cc. of evaporated urine; the dry residue was transferred to an allundum thimble and extracted in a Soxhlet apparatus for four hours with chloroform as solvent. Titration was then performed with decinormal sodium ethylate from sodium, as in the case of the hydrolysed hippuric acid determinations. On the thirty-fourth day the dogs were killed by means of coal gas and autopsied.

RESULTS.

The following table gives the results for the two dogs extending over a period of 34 days; the quantities are calculated for 24-hour periods:

Day	Dog	Hippuric N.	Amino N.	NaCl	24 hour quantity urine
1	1	0.014 mg.	0.035 g.	1.45 g.	150 cc.
	2	0.023	0.749	0.96	130
2	1	0.026	4.190	3.07	360
	2	0.049	2.120	0.28	300
3	1	None	None	None	None
	2	0.029	"	0.58	100
4	1	0.014	0.190	5.90	100
	2	0.040	0.888	0.25	550
5	1	0.010	0.190	1.40	150
	2	0.026	0.526	0.19	275
6	1	None	None	None	None
	2	0.119	2.15	0.39	500
7	1	0.083	3.51	2.80	500
	2	0.037	0.800	0.25	500
8	1	None	None	None	None 2 g. sodium benzoate
	2	"	"	0.12	400

Day	Dog	Hippuric N.	Amino N.	NaCl	24 hour quantity urine
9	1	0.174	3.05	3.20	425
	2	0.091	None	0.51	225
10	1	0.030	1.05	1.70	250
	2	0.076	0.945	0.28	450
11	1	0.054	1.91	2.10	230
	2	0.040	0.314	0.27	260
12	1	0.058	2.22	3.50	400
	2	0.074	1.860	None	380 500 mg. hydrazine sulphate per os.
13	1	None	None	"	None
	2	"	"	0.17	375
14	1	"	1.70	1.40	250
	2	0.089	1.97	0.26	400
15	1	0.010	0.124	1.20	140
	2	0.132	0.895	0.26	500
16	1	0.100	1.43	3.50	450
	2	0.29	1.87	0.14	260
17	1	None	None	None	None
	2	0.017	0.776	0.16	250
18	1	0.071	None	3.20	450
	2	0.026	"	0.15	280
19	1	None	0.22	3.60	500
	2	0.020	0.717	0.22	400 500 mg. hydrazine sulphate per os.
20	1	0.010	1.00	None	150
	2	0.013	1.134	"	160
21	1	None	None	0.50	200
	2	"	"	0.12	180
22	1	0.018	"	0.51	150
	2	0.028	1.05	0.41	150
23	1	0.024	1.37	None	180
	2	0.024	None	"	350
24	1	None	"	0.82	130
	2	"	"	0.18	150
25	1	0.036	"	0.37	450
	2	None	"	0.21	250 500 mg. hydrazine sulphate sub-cut.
26	1	0.016	0.504	0.92	250
	2	0.018	1.091	0.37	150 2 g. sodium benzoate
27	1	0.099	1.74	0.45	250
	2	0.051	1.728	None	250
28	1	0.020	1.740	0.26	200
	2	None	None	0.13	225
29	1	0.028	2.33	None	375
	2	0.015	0.981	"	130
30	1	0.014	0.733	"	100
	2	0.042	2.52	"	325
31	1	0.010	None	0.83	100
	2	0.026	2.106	None	300
32	1	None	None	1.73	350
	2	"	"	0.072	225 500 mg. hydrazine sulphate sub-cut.
33	1	0.013	0.075	0.75	160
	2	None	0.114	0.10	200
34	1	0.017	None	0.43	300
	2	0.014	0.353	0.07	250 2 g. sodium benzoate

Professor O. T. Schultz, who kindly performed the autopsies and examined the slides from formalin and Zenker fixations of portions of the livers and kidneys of the two dogs, reports that both dogs showed marked fatty degeneration of the liver, while the kidneys were intact.

DISCUSSION.

We may summarise the results by averages of the amounts of the various substances excreted during the different periods of the experiments.

Average of excretions previous to administration of sodium benzoate or hydrazine:

Dog	Hippuric N.	Amino N.	Chlorides	Quantities
1	0.031	1.98	2.92	252
2	0.043	1.17	0.45	332

Average after giving 2 g. of sodium benzoate:

1	0.085	2.00	2.33	301
2	0.069	0.629	0.29	301

Average after giving hydrazine sulphate by mouth:

1	0.059	1.36	2.48	323
2	0.104	1.47	0.019	349

Average after a second feeding of hydrazine sulphate:

1	0.017	0.863	1.35	218
2	0.021	0.969	0.23	231

Average after injection of hydrazine subcutaneously:

1	0.026	0.50	0.64	350
2	0.018	1.091	0.29	200

Average after feeding sodium benzoate following hydrazine injection:

1	0.040	6.54	0.51	203
2	0.033	1.83	0.13	246

Average after a second injection of hydrazine:

1	0.013	0.075	1.24	255
2	None	0.57	0.086	213

Average after a final feeding of benzoate following hydrazine injection:

1	0.017	None	0.43	300
2	0.014	0.353	0.07	250

From these results, we see that following the feeding of sodium benzoate initially after establishing the normal excretion, the hippuric acid nitrogen is increased. This is true after we subtract the free benzoic acid figure from the total amount determined after hydrolysis, namely, 0.026, leaving 0.065 mg in the case of Dog 1. After the feeding of hydrazine by mouth, upon the first

occasion, there is no apparent disturbance of hippuric acid excretion, such as we find in the case of the second feeding. There is an apparent reduction of the amount of hippuric acid after the injection of hydrazine subcutaneously, but inasmuch as we find a rise in excretion following the administration of sodium benzoate, we may conclude that no extensive damage has been done to the liver up to this point. After a second injection of hydrazine subcutaneously, there is a marked fall in the case of Dog 1, the figures for the second dog being lost due to defect in the water system passing through the reflux condensers during hydrolysis. Finally, after the feeding of sodium benzoate after the second injection of hydrazine, a marked fall in hippuric acid excretion is observed. Obviously, we are not able to say how great a difference in liver involvement has occurred between the twenty-fifth day and the final day of the experiment, but from the figures, it is evident that the damage has been progressive.

During these periods, the kidneys have remained intact, as we may observe from the chloride excretion; in studying these figures, it must be borne in mind that during the last periods of the experiments, the dogs took less and less food, so that the chlorides appeared in progressively smaller amounts. However, a comparison of the figure for the chlorides excreted by Dog 1 during the first period and during the next from the last, namely, a comparison of 2.92 g. with 1.24 g., makes it evident that after it has become established that the liver is damaged, the kidneys are excreting quantities of chlorides not far from what we should expect, taking into consideration the lessened intake in the food. The chemical figures are fully borne out by the study of the histological sections.

We were disappointed in finding that the amino-nitrogen could not be correlated with the hippuric acid figures. At best, the glycocoll fraction is not large and might easily be overbalanced by changes in the amino-nitrogen fraction due to the degenerative changes of the liver. Henriques and Sørensen [1909] have utilised the glycocoll fraction after hydrolysis of hippuric acid, the determination being made by means of the formaldehyde-titration method.

From the figures representing the 24 hour quantities of urine, it is apparent that there is no effect either as diuretic or anuretic exerted by the reagents used in the experiments. Consequently, the results are not vitiated by these factors, which Astolfoni [1905] found operative in his experiments, hippuric acid being excreted in increased amounts following the administration of diuretics and vice versa.

The figures obtained upon free benzoic acid in the urine show that there is

no effect upon the relative amounts of hippuric acid estimated as benzoic acid after hydrolysis, by this factor, so that the criticism of Lewiniski [1908] cannot be applied to this work.

The conclusion that the liver plays an important role in hippuric acid synthesis is well in keeping with what we know of the functions of the liver, as contrasted with those of the kidney. There are a number of conjugations performed in the liver. Thus, various syntheses are known involving the acids, such as phosphoric acid, sulphuric acid, glycuronic acid, etc. Conjugations with sulphuric acid lead to the formation of the so-called ethereal sulphates; pairing of glycuronic acid with substances of various sorts leads to the formation of glucoside compounds. Conjugation of glycocoll occurs with any compound which after oxidation leads to the formation of benzoic acid (salicylic acid, *p*-hydroxybenzoic acid, etc.), the end-product being the so-called "ur-acids" of acid-amino nature. In birds, the formation of dibenzoylornithine, where ornithine takes the place of glycocoll in hippuric acid syntheses, is known to take place principally in the liver. In the dog, a subsequent pairing of hippuric acid and urea takes place in the liver. This does not necessarily show that the primary conjugation to form hippuric acid occurs in the same organ as the secondary pairing, yet there is reason to believe that this is the case. The alleged hippuric acid synthesis is the exception to the rule concerning one of the characteristic functions of the liver, if we adopt the conclusions of Bunge and Schmiedeberg and their school as final.

CONCLUSIONS.

Data are presented to show that the liver participates in hippuric acid conjugation to a marked extent. The data have been derived by poisoning the liver with hydrazine, leaving the kidneys intact. The conclusions reached in this investigation are borne out by the work of Kingsbury and Bell and are aligned with our knowledge of the characteristic synthetic functions of the liver as contrasted with those of the kidney.

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