V. CARNOSINE OF MUSCLE AND IMINAZOLE EXCRETION IN THE URINE.

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ONE of the conclusions in a recent communication by the writer was that the carnosine content of striated muscle in the cat fluctuates [Hunter, 1924]. It was shown that the carnosine content of white muscle could be lowered; it is now indicated that it can be lowered or raised. Lack of exercise was suggested as a possible cause of the fall in carnosine values shown to occur in certain caged animals. Further work has shown that diet has a definite influence on the carnosine content of muscle, although it has not been disproved that exercise plays a part.

It was previously indicated that a rapid fall in the carnosine content of muscle might be accompanied by an increased iminazole excretion in the urine. This hypothesis appeared true when it was found that two cats, with a high carnosine value in the muscle of an amputated leg, passed, during the following 24 hours—when presumably the muscles were rapidly losing carnosine—urines which showed a more than usually red colour with the diazo reagents.

On the publication of a method for the estimation of iminazoles in urine by Koessler and Hanke [1924] it was decided to test this hypothesis further. The results reported here are evidence in its favour.

The estimation of iminazoles in the urine is of recent date, the first attempt perhaps being made by the writer in 1922 [Hunter, 1922, 1]. It was then shown that a litre of a mixed sample of urine from patients suffering from measles contained 310 mg. iminazole calculated as histidine monohydrochloride, and approximately 100 mg. of the pure substance were isolated and identified.

The recent method of Koessler and Hanke is suitable for the routine estimation of iminazoles in urine. The use by these workers of sodium hydroxide along with lead acetate is more satisfactory than that of baryta as used by the writer. The subsequent evaporation of the filtrate is also indispensable.

EXPERIMENTAL.

1. *Changes in the Carnosine Content of Muscle.*

In Table I is shown the carnosine content of the muscles, gastrocnemius, plantaris and soleus, in the amputated limbs of five cats, and after a varying interval the carnosine content of the corresponding muscles of the opposite limbs. The first date in each case is the day on which the right leg was amputated and the carnosine content of the muscles determined; the second date, the day on which the cat was killed and the carnosine values of the corresponding muscles of the left leg estimated.

Table I.

Cat No.	Date	Wt. of cat g.	Gastrocnemius		Plantaris		Soleus	
			Wt. of muscle g.	% carnosine	Wt. of muscle g.	% carnosine	Wt. of muscle g.	% carnosine
13	26. ii. 24	2681	17.870	0.356	5.184	0.298	3.030	0.051
	2. iv. 24	2710	17.790	0.431	4.859	0.288	3.270	0.050
15	26. iii. 24	1915	13.055	0.131	4.349	0.097	1.710	0.065
	7. iv. 24	1740	11.975	0.023	—	—	1.450	0.064
18	11. vi. 24	2100	13.370	0.014	4.230	0.014	2.212	0.087
	20. vii. 24	3170	22.770	0.258	7.030	0.214	3.500	0.052
Lateral gastrocnemius								
19	2. vi. 24	3170	9.480	0.386	6.140	0.389	2.890	0.057
	5. vi. 24	2810	8.170	0.166	5.520	0.163	2.900	0.051
Lateral gastrocnemius								
20	31. v. 24	3168	11.195	0.286	7.519	0.262	3.507	0.082
	12. vi. 24	2750	8.070	0.016	4.534	0.022	3.315	0.084

Cat No. 13 belonged to the same litter as cats Nos. 5 and 6 of a previous paper [Hunter, 1924]. Cats Nos. 5 and 6 gave very low values for the gastrocnemius, viz. 0.01 % and 0.007 % and cat No. 13 would probably have given like values had it been killed at the same time. It was however fed on meat until it was in a moderately good condition. When the right leg was amputated the gastrocnemius contained 0.356 % carnosine. The weight of the cat after the amputation was 2681 g. It recovered well although it lost weight for the following two weeks. A good meat diet was given and the weight increased progressively, reaching 2710 g. when the animal was killed, about 5 weeks after the operation. The weights of the two gastrocnemii were practically the same, but the second carnosine value was 0.075 % greater than the first. This increased carnosine value, however, is not borne out in the values from the plantaris. The soleus values remained, as usual, constant. Owing to the cat's loss of weight during the two weeks following the amputation the carnosine content of the gastrocnemius probably fell during that period.

That the carnosine content of muscle may be increased is clearly shown in the case of cat No. 18. This animal was starved for 15 days during which time

its weight fell from 3000 to 2100 g. The carnosine content of the gastrocnemius at the end of the starvation was 0.014 %. For the next three weeks the cat was kept on a maintenance diet of meat and during the following three weeks was given a larger amount so that the weight increased to about 1000 g. more than at the date of amputation. (Further data on this cat are given in Table II.) The carnosine content of the gastrocnemius was now 0.258 %, of the plantaris 0.214 %, and of the soleus 0.052 %. The change in the value from the soleus is unusual.

Cats Nos. 15, 19 and 20 were starved after the operation. In all cases the carnosine values of the white muscles fell.

It may be concluded from the results in Table I that the carnosine content of striated muscle falls during starvation and increases after a meat diet if the weight of the cat increases, even when cats are caged. It is thus clear that if exercise influences the carnosine content of muscle it is not a controlling factor.

2. *Urinary Iminazole of Cats.*

When cats are on a constant diet their daily urine output is fairly regular; and, since they show probably a wider variation in the carnosine content of their muscles than any other laboratory animal, they are peculiarly suitable to use in order to determine whether there is an increased iminazole output in the urine when the carnosine content of the muscle falls rapidly. The iminazole in the urine was estimated according to a modified Koessler and Hanke [1924] procedure. (This modification it is hoped to deal with in detail in a forthcoming paper.) The total daily output of urine was used for each estimation with proportionate amounts of lead acetate and sodium hydroxide. The values are given as "iminazole" according to the suggestion of Koessler and Hanke.

The five cats reported in Table II were all in excellent condition when the urine examination was begun and the carnosine content of their muscles was probably high.

It is notable that with the exception of cat No. 19 the urine on the first day of starvation showed the highest iminazole content over the periods recorded. (Cat No. 21 may be regarded as starving, as it would not at first eat the bread after a diet of meat.) There tends also to be an increased iminazole excretion following amputation, especially so in cats Nos. 19 and 20, where the carnosine value in the muscle is shown to be high. This is less marked in cat No. 18, where the value in the amputated limb was low. The value of 6.0 mg. in this cat following amputation represents two days' output of urine; in such cases the value is generally about double, even if the volume is little changed. This feature is again salient in the same cat on June 14th and 27th, and in cat No. 21 on June 23rd.

The urine from cat No. 17 was not examined over a sufficiently long period to provide conclusive evidence of a fall in iminazole output, as the cat unex-

Table II.

Cat no. 17.

Date	Urine cc.	Iminazole mg.		Diet and remarks
May 29	64	12.5	↑ Starvation ↓	Wt. of cat 3520 g.
" 30	none	—		
" 31	41	7.8		
June 1	none	—		
" 2	38	6.8		
" 3	none	—		
" 4	47	7.5		1 hour under chloroform
" 5, 6	none	—		
" 7	47	6.5		
" 8	65	9.7		

Cat died on June 9th. Wt., June 9th, 3050 g.
Carnosine: r. plantaris 0.181 %; l. plantaris 0.184 %.

Cat no. 18.

Date	Urine cc.	Iminazole mg.		Diet and remarks
May 28	105	12.5	↑ Starvation ↓	Wt. 3000 g.
" 29	none	—		
" 30	73	8.2		
" 31	none	—		
June 1	83	9.4		
" 2	none	—		
" 3	57	6.0		
" 4	35	3.5		
" 5	49	4.2		
" 6	none	—		Given 200 mg. carnosine intravenously
" 7	45	21.5		
" 8	33	3.2		
" 9	25	1.2	↓ 90 g. meat per day ↑	Wt. of cat 2100 g.; carnosine 0.014 % (gastrocnemius)
" 10	45	1.3		
" 11	none	—		
" 12	78	6.0		
" 13	none	—		
" 14	78	10.8		
" 15	38	8.0		
" 16	52	7.6		
" 17	42	4.6		
" 18	45	4.4		
" 19	43	4.8		
" 20	30	4.0		
" 21	42	4.5		
" 22	41	5.3		
" 23	40	5.2		
" 24	36	4.3		
" 25	40	3.9		
" 26	none	—		
" 27	55	8.5		
" 28	41	6.1		
" 29	42	7.0		
" 30	39	6.1		
July 1	lost	lost		Wt. of cat 2150 g. Given 500 mg. carnosine orally
" 2	40	7.2		

Cat fed from July 2nd till killed, July 20th, on 200 g. of meat per day.
Wt., July 20th, 3170 g.; carnosine 0.258 % (gastrocnemius).

Cat no. 19.

Date	Urine cc.	Iminazole mg.		Diet and remarks
May 27	35	7.9	↑ Starvation	Wt. 3680 g.
" 28-30	none	—		
" 31	35	9.0		
June 1-2	none	—		June 2nd, carnosine 0.386 % (lat. gast.)
" 3	35	14.0		
" 4	16	4.5		

Cat died June 5th. Wt., June 5th, 2810 g.; carnosine 0.166 % (lat. gast.)

Cat no. 20.

Date	Urine cc.	Iminazole mg.		Diet and remarks
May 31	108	24.2	↑ Starvation	
June 1	none	—		Wt. of cat 3670 g.; carnosine 0.286 % (lat. gast.)
" 2	140	22.0		
" 3	none	—		
" 4	50	7.2		
" 5	66	6.3		
" 6	40	3.0		
" 7	35	3.2		
" 8	32	3.0		
" 9	none	—		
" 10	32	2.2		
" 11	45	4.5		

Cat killed June 12th. Wt., June 12th, 2750 g.; carnosine 0.016 % (lat. gast.)

Cat no. 21.

Date	Urine cc.	Iminazole mg.		Diet and remarks
June 14	84	12.7	↑ Bread and water and little milk	Some faeces in urine
" 15	157	4.0		
" 16	60	8.0		
" 17	132	6.5		
" 18	92	4.2		
" 19	121	4.5		
" 20	175	4.8		" " " "
" 21	175	3.0		" " " "
" 22	none	—		" " " "
" 23	105	7.8		
" 24	170	7.0		
" 25	168	4.2		
" 26	214	5.7		" " " "
" 27	150	2.5		
" 28	80	2.4		
" 29	181	4.0		
" 30	98	5.5		
July 1	163	2.5		
" 2	178	3.6		

Cat kept on above diet till Aug. 4th, when killed. Wt., Aug. 4th, 1860 g.; carnosine 0.023 %.

pectedly died after 10 days' starvation, showing a loss of about 500 g. in weight. At death the carnosine content of the corresponding plantaris muscles was 0.181 and 0.184 %. Even taking account of the irregularity of the urine output the values are relatively high and would probably have fallen after the remaining carnosine had left the muscle.

The iminazole values for cat No. 18 show a fairly progressive fall during the starvation period. For a few days following amputation there is a slight rise in values, after which, for a period of nine days, there is a very regular urine output and the iminazole values show little variation. During the last week of the urine examination the iminazole output again rises owing to an increase in the muscle carnosine following a meat diet.

Cat No. 19 serves to demonstrate a high iminazole output immediately following amputation. This cat also died for unknown reasons after the loss of about 800 g. in weight, the gastrocnemius still showing 0.166 % carnosine. The values as in cat No. 17 are higher than usual throughout.

Cat No. 20 shows a progressive fall in the iminazole of the urine as starvation continues. Over a period of 11 days the carnosine content of the gastrocnemius falls from 0.286 to 0.016 %.

Cat No. 21 was maintained on a diet of bread and water with a little milk added. The urinary output is very high and relatively constant and the iminazole values show some fluctuation. During the first 10 days, however, the values are as a whole higher than in the succeeding period.

During the starvation period, on June 6th, cat No. 18 was given 200 mg. carnosine intravenously. The urine on the following day showed a decided rise in iminazole, representing about one-tenth of the administered carnosine. On July 1st, when on a meat diet, this same cat was given 500 mg. of carnosine sprinkled over the surface of the meat which the cat ate under observation. The urine on the following day showed no marked rise in iminazole output. It is thus clear that a cat can ingest relatively large amounts of carnosine without increase in iminazole output. It is less clear how the cat deals with iminazole actually in the blood stream. For the carnosine was given intravenously at a single injection and at least part of the 21.5 mg. recorded was carnosine as shown by a decrease in colour value on hydrolysis and an increased Knoop test [Hunter, 1922, 2]. However 200 mg. of carnosine is probably not an abnormal amount to be thrown into the circulation on a single day in a cat whose muscle is rapidly losing carnosine—assuming that the carnosine lost by muscle enters the blood stream. For as shown in cat No. 19, the carnosine content of the gastrocnemius fell to the extent of 0.22 % in three days, representing a little over 0.7 g. per kg. of muscle per day. Taking the muscle mass of cat No. 19 as 1400 g. this indicates a loss from the total muscle of rather more than 0.9 g. carnosine. Allowing for red muscle and other muscle with less carnosine than the gastrocnemius, it seems not unlikely that at least 500 mg. may be dealt with by the blood stream per day from this source alone.

The balance of evidence from the urines of the five cats represented in Table II dips in favour of the view that some of the urinary iminazole comes from the carnosine of muscle. Yet there are irregularities in the iminazole excretion which cannot be accounted for on this assumption. Perhaps a clear proof is not to be expected, for, if carnosine is a reserve material, it is probably in large part utilised by the starving animal. Further, when carnosine rapidly

disappears from muscle only a fraction may reach the blood stream, and of this fraction only a part of the carnosine or its breakdown products is likely to reach the urine with the iminazole ring intact. An increase in the iminazole content of the blood will require to be demonstrated before it can be determined whether the carnosine lost from muscle enters the blood stream or is directly utilised by muscle. There is not yet a suitable method for the estimation of iminazoles in small quantities of blood. The writer has found by a fractional precipitation that there are at least 1 to 2 mg. of carnosine per 100 cc. of ox blood.

An attempt was made in several of the cat urines to determine the main iminazole present. In one cat the lead acetate filtrates were collected and precipitated by mercuric acetate. Nearly all of the colour giving substance was precipitated in this manner. The substance was also precipitated in a *slightly acid solution* by silver nitrate. The final solution obtained after several precipitations in this manner gave a colour which developed more quickly and was finally more red than that obtained from histidine. The solution of the substance was strongly acid to litmus and rapidly decolorised alkaline permanganate. It gave a strongly positive Knoop test.

Its behaviour in the silver precipitation, its acid reaction, and its unsaturation parallel the properties of urocanic acid. It is not stated in the literature whether urocanic acid gives a positive Knoop test and its colour with the modern diazo reagents has not been compared with that obtained from histidine.

It was estimated on an arbitrary molecular colour value of 130 million mm. that there was about 0.2 g. of the substance in the solution above referred to, yet crystals could not be obtained directly in the solution, nor with picric or nitric acids. It might be supposed from the literature on urocanic acid that at least a picrate should have been obtained under the conditions were the substance urocanic acid¹.

¹ Through the courtesy of Professor Andrew Hunter of this University the writer has obtained some pure urocanic acid. A saturated solution of this substance (about 0.2 % at 20°) is quite negative to Knoop's test. Its behaviour with the diazo reagents is also noteworthy. A solution containing 0.04 mg. per cc. of $C_6H_6O_2N_2 \cdot 2H_2O$, when tested for histidine and carnosine, as described in the paper which follows, gives a molecular colour value of approximately 109 million mm. The final colour developed by urocanic acid with the diazo reagents is, however, more yellow than that produced by histidine and its time of colour development is much more prolonged, viz. 12-14 mins. The intensity of colour produced is proportional to the amount of urocanic acid present.

Although the substance from cat's urine above described is acid in reaction, unsaturated and gives a colour like iminazoles with the diazo reagents, it is most probably not urocanic acid because it is much more soluble in water, it gives a Knoop test and behaves differently with the diazo reagents as regards time of colour development and shade of colour.

SUMMARY.

1. The carnosine content of striated muscle in the cat can be lowered by starvation, and if lowered can again be raised by a meat diet.

2. When carnosine disappears rapidly from muscle there is evidence of an increased excretion of iminazole in the urine.

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