LIV. OBSERVATIONS ON THE DISTRIBUTION AND VARIATION OF CARNOSINE IN CAT MUSCLE.

BY GEORGE HUNTER,

Department of Pathological Chemistry, University of Toronto.

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IN a previous paper [1922] it was shown that the carnosine content of muscle varies with the species of animal and with different members of the same species. The findings reported here deal only with cat muscle, and confirm previous observations. They further show that in the same animal carnosine is a variable constituent of white muscle, but is approximately constant in red.

Table I shows the carnosine content of several muscles of the right and left legs of six cats. The first value given in each case is for the right leg. The weights of cats Nos. 1, 2 and 5 were not recorded.

Table I.

Cat No.	Weight g.	Gastrocnemius % Carnosine	Soleus % Carnosine	Biceps femoralis % Carnosine	Semimembranosus % Carnosine	Semitendinosus % Carnosine	Vastus intermedius % Carnosine
1	—	0.357	0.051	0.494	0.492	0.294	—
		0.350	0.053	0.483	0.490	0.278	—
2	—	0.251	0.067	—	—	—	—
		0.257	0.059	—	—	—	—
3	2680	0.038	—	—	—	—	—
		0.039	—	—	—	—	—
4	2780	0.037	—	—	—	—	—
		0.039	—	—	—	—	—
5	—	0.010	0.055	0.014	0.014	0.009	0.038
		0.010	0.062	0.013	0.011	0.009	0.032
6	1470	0.007	0.049	0.012	—	—	—
		0.007	0.048	0.011	—	—	—

It may be seen from the above table that (a) corresponding muscles in the same cat have the same carnosine content; (b) the carnosine content of the gastrocnemius, biceps femoralis, semimembranosus and semitendinosus is subject to wide variation in the different cats; (c) the carnosine content of the soleus muscle is constantly low and subject to smaller variation; (d) red muscle may have a lower or higher carnosine content than white muscle.

It may further be seen that the weight of the cat has no relation to the carnosine content of its muscle. Although not weighed, cats Nos. 1 and 2 were certainly smaller animals than 3 and 4, as could be judged from the weights of the whole muscles which were taken for each estimation. The significant difference between these two pairs of cats was that Nos. 1 and 2 were killed shortly after arrival at the laboratory, whereas Nos. 3 and 4 were

kept in cages for ten days. During this period they each consumed 60 g. of raw meat per day.

Cat No. 5 was very lean, having been partially starved during the week previous to killing. Cat No. 6 was starved for ten days in a cage, and was very weak before killing. That inanition does not wholly account for the low values obtained in cats Nos. 5 and 6 is indicated by the fact that the values obtained in cats Nos. 3 and 4 were likewise very low, although the cats were in excellent condition.

It was suspected that confinement might affect the carnosine; at any rate it was desirable to determine whether it was a fluctuating or constant constituent of muscle.

The procedure adopted was to amputate a leg, under chloroform, estimate the carnosine in various muscles, kill after several days and estimate the carnosine in the corresponding muscles of the other leg.

That the carnosine content did not fall during the actual period of anaesthesia was shown in the following way. A cat was anaesthetised with ether, and the right leg quickly severed near the middle of the femur. Chloroform was then substituted as anaesthetic and the animal kept under for 75 minutes. At the end of this time the animal was killed with the anaesthetic. The gastrocnemius and soleus were then tested in the corresponding legs, with results as follows:

Gastrocnemius before chloroform	0.251 % carnosine
Gastrocnemius after chloroform	0.257 "
Soleus before chloroform	0.067 "
Soleus after chloroform	0.059 "

Four cats were treated as described above, and the results are given in Table II. The first date in each case is the day on which the right leg was amputated; the second, the day on which the animal was killed and the muscles of the left leg estimated. The weights of the individual muscles are given in order that the degree of wastage may be noted.

Table II.

Cat No.	Wt. before operation g.	Date	Gastrocnemius		Plantaris		Soleus	
			Wt. of muscle g.	% Carnosine	Wt. of muscle g.	% Carnosine	Wt. of muscle g.	% Carnosine
7	2480	28. xi. 23	14.539	0.185	4.453	0.161	2.423	0.085
		7. xii. 23	11.660	0.054	3.703	0.048	2.138	0.090
8	2470	11. xii. 23	14.848	0.363	4.794	0.349	2.354	0.054
		22. xii. 23	11.159	0.020	3.236	0.015	1.945	0.053
9	2740	14. xii. 23	22.930	0.433	6.502	0.350	2.393	0.058
		24. xii. 23	16.839	0.020	4.719	0.015	2.083	0.059
10	2190	24. xii. 23	13.549	0.320	4.294	0.244	1.894	0.054
		28. xii. 23	11.879	0.164	3.534	0.094	1.820	0.053

Cats Nos. 8 and 9 were in the cage only a few hours before operation; cat No. 7 for three days, and cat No. 10 for ten days.

In all cases a good recovery was made after the operation. Cats Nos. 7 and 10 ate raw meat on the second day; cats Nos. 8 and 9 were slower in regaining their appetite.

Table II shows that (a) in the space of ten days the gastrocnemius and plantaris in cats Nos. 7, 8 and 9 dropped from one-quarter to one-twentieth of their original values, and that in the space of four days the values in cat No. 10 dropped to less than half; (b) the carnosine content of the soleus did not vary in the same cat, and varied only slightly in the different cats.

That carnosine varies in different muscles of the cat has recently been shown by Mitsuda [1923], who has also confirmed the writer's original observation that carnosine varies in the muscle of different animals of the same species. In the light of the results now reported this is to be expected. As has been shown, carnosine varies within wide limits in the same muscle of the same animal. As regards the lower limit, it is probable that it never entirely disappears. Even in cat No. 6, which was on the point of dying from starvation before it was killed, the gastrocnemius had 0.007 % carnosine¹.

One cannot be certain about the upper limit, but it seems highly improbable that even the semimembranosus reaches the value of 1.799 % carnosine as given by Mitsuda². The highest value the writer has found in a gastrocnemius was in cat No. 9, *i.e.* 0.433 %. It is probable that the semimembranosus was between 0.5 % and 0.6 % in this case.

It is seen that there is a fairly constant relationship among the muscles which vary in their carnosine content. The semimembranosus and biceps femoralis have approximately the same values. Both are higher than the gastrocnemius, which in turn is slightly higher than the plantaris. The semitendinosus is still lower than the plantaris, and it may be noted that this relationship holds whether the carnosine content be high or low.

The results are more suggestive than enlightening. They suggest that the carnosine content of muscle falls with confinement or want of exercise. Even when the cats were fed on butcher's meat the values continued to fall. The difficulty experienced by some in the preparation of carnosine may be in part explained by the fact that some butcher's meats have a low carnosine content. The writer has found ox muscle with less than 0.1 % carnosine. It is possible

¹ This is not a very reliable figure, because the azo colour developed in such conditions is very yellow; yet it is remarkably proportional, and has about the same developmental time as pure carnosine solutions. The relatively greater amount of potentially interfering substances may easily account for the yellow colour [see Hunter, 1922].

² There is either an error or a misprint in Mitsuda's paper. He makes a standard carnosine solution containing 0.00001 g. of carnosine nitrate in 1 cc., of which he states 0.3 cc. contains 0.03 mg. ! Does this mean that his values are only one-tenth of those published?—in which case they would represent cats with rather low but more probable values. That the discrepancies between our findings are due to an error in standards or calculations is more probable than that they are due to actual differences in different animals. The almost constant values obtained for the soleus of different animals point to this conclusion.

It would seem desirable that future workers on carnosine should give the molecular colour value—as measured against Koessler and Hanke's [1919] colour standard for histidine—of the carnosine used as standard. If this were done there would then be a definite standard of comparison among the results of different workers, and one could perhaps trace the source of discrepancies. While in Prof. Cathcart's laboratory in Glasgow, the writer published his molecular colour value as measured from carnosine, and from carnosine nitrate. He has again prepared carnosine since coming to Canada, has reconfirmed his previous results and further reinforced these by measurement of the molecular colour value from carnosine-copper. [Clifford, 1921; Mitsuda, 1923; Hunter, 1921, 1922.]

that the muscle of stall-fed cattle has a lower carnosine content than that of animals grazed in the open.

Why there should be a fairly constant relationship in the carnosine content of those muscles with fluctuating values, or why the soleus content should be low but constant, is quite obscure. It would be premature to say that carnosine is unique in this manner of variation, as very little attention appears to have been given by physiologists to the chemistry of individual muscles. Consideration of the function, blood or nerve supply of the various muscles offers no obvious explanation.

Mere mention may be made here of the behaviour of the urines towards the diazo reagent in these cases. One might suspect that some carnosine or derivative of that substance would appear in the urine when carnosine rapidly disappears from muscle. Indications of this have indeed been obtained. A further report on this subject is pending.

SUMMARY.

1. Corresponding muscles in the cat have the same carnosine content.
2. The carnosine content of the same white muscles in different cats is subject to wide variation. This confirms Mitsuda's finding, although the absolute values obtained by Mitsuda are questioned. In the gastrocnemius, the highest and lowest values found were 0.433 % and 0.007 % carnosine.
3. The carnosine content of red muscle, *e.g.* soleus, of different cats is always low, and subject only to slight variation. The highest and lowest values found were 0.090 % and 0.048 % carnosine.
4. The carnosine content of white muscle in the same cat fluctuates. Lack of exercise is suggested as a possible cause.
5. The carnosine content of red muscle in the same cat, *e.g.* soleus, does not show this fluctuation.
6. There is a fairly constant relationship between the carnosine contents of the fluctuating muscles, whether the values be high or low.
7. The same muscles in different cats show as wide a variation as different muscles in the same cat. The carnosine content of red muscle is not necessarily lower than that of white muscle. In these two points the writer differs from Mitsuda.

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