LXXX. THE DISTRIBUTION OF CARNOSINE IN THE MUSCLES OF THE NORMAL AND DECEREBRATE CAT.

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CARNOSINE is noteworthy as being the only tissue constituent containing a β -amino acid. Its biological significance is as yet obscure. The work described in the present paper forms part of an endeavour to decide whether the base suffers a change of concentration during changes in the metabolic conditions of muscles.

An entirely satisfactory method for its estimation is yet lacking. Skworzow first attempted quantitative determination of carnosine by estimating the nitrogen content of the carnosine silver fraction from muscle extract. Mauthner [1913] unsuccessfully attempted the quantitative isolation of carnosine as the copper salt which had been prepared previously by Gulewitsch. Fürth and Hryntschak [1914] first attempted to found a colorimetric estimation of carnosine nitrate on the fact that histidine when treated with diazobenzene sulphanilic acid and sodium carbonate, gives rise to an intense red colour. But the results given by these authors show that the method was decidedly inaccurate. Koessler and Hanke [1919] described a colorimetric method for the estimation of iminazole derivatives based on the diazo reaction and claimed a fair degree of accuracy and facility for the method. The chief modification introduced by them was the substitution of the standard histidine solution by a standard consisting of a mixture of methyl-orange and Congo-red ("CR . MO"), which gave a colour matchable with diazotised histidine. Clifford [1921] suggested a further modification of Koessler and Hanke's method. The latter authors obtained a perfect linear relationship between the amounts of histidine diazotised and the reading of the standard (CR . MO) cylinder. Clifford, however, using standard solutions of histidine and carnosine matched against a CR . MO indicator solution found that linear relationship between depth of colour and amounts of carnosine diazotised did not hold.

On the other hand, Hunter [1921] a little later confirmed Koessler and Hanke's statements.

Hunter found, however, that solutions containing carnosine, after heating to 100° for one hour, yielded less colour than the original solution, and further that filtration through three filter papers in succession caused a loss of 44 %

of the carnosine present. He also declared there is reason to believe that part of the colour obtained is due to substances other than carnosine present in the muscle extract. Proteins had been found by Koessler and Hanke to lower the reading of a standard histidine solution by 30 %.

It is clear, then, that the colorimetric estimation of carnosine is not yet placed upon a satisfactory basis, but requires further study.

According to Clifford [1921] carnosine is absent from the flesh of all invertebrates, white and flat fishes, chelonians, and birds belonging to the finch and owl tribes, but is found in fishes rich in fat, and most reptiles and amphibians, in birds, and in all mammals. The amount present is practically constant in individuals of any one species.

After keeping the muscles of mammals and fishes under ordinary conditions for four days after death, Clifford found no decrease in amount of carnosine originally present, but putrid meat yielded smaller amounts.

The claim of Pekelharing and his co-workers that in the metabolism of Muscle Tonus there is an increase of creatine seemed to make it desirable to determine if other nitrogenous metabolites in the tissue undergo similar changes and the research here reported deals with the carnosine contents of the leg muscles of cats under normal condition and in decerebrate rigidity respectively. The determination was carried out chiefly by Clifford's modification of Koessler and Hanke's method. The author has, however, slightly modified this method as will be seen from the following description.

Method of Extraction employed.

5-10 g. of finely divided muscle were placed in an Erlenmeyer's flask of about 300 cc. capacity and extracted with 100 cc. of distilled water at 70° for 30 minutes, the extract being poured into a 500 cc. flask. The residue was ground to pulp and extraction as above was repeated three times. Finally the residue was transferred to the half-litre flask and the whole made up to about 450 cc. with distilled water. The extraction was allowed to continue for one hour at room temperature with frequent shaking, and the flask was then heated 30 minutes at 100°. After cooling, the contents of the flask were made up to the 500 cc. mark with water and then filtered. The first 20 cc. or so of the filtrate were rejected. Of the main bulk 50 cc. were placed in a dry flask and 10 cc. of a 20 % solution of metaphosphoric acid were added to precipitate the soluble protein which otherwise prevents the colour development of carnosine. The mixture was left to stand 2-24 hours.

The solution was then filtered from the precipitated protein through a dry filter paper into a dry beaker. About 20 cc. of the first filtrate were again rejected to avoid any lowering of the result due to absorption by the filter paper. 30 cc. (corresponding with 25 cc. of the original extract) were placed in a 50 cc. volumetric flask and neutralised to litmus paper with 10 % caustic soda solution and made up to the mark with distilled water. Of this filtrate 0.1-1.0 cc. was used for the colorimetric determinations. The amount thus

measured (x cc.) was diluted with $(1 - x)$ cc. of distilled water; $x/2$ cc. will be seen to represent the corresponding amount of the original extract.

Standardisation of Standard Indicator. A solution of carnosine nitrate containing 0.00001 g. of the salt in 1 cc. was used as a primary standard for the preparation of the Congo red-methyl orange solution which was employed as the permanent standard. It was diazotised in one of the vessels of a Duboscq's colorimeter. Placing $(1 - x)$ cc. of distilled water, 5 cc. of 1.1 % sodium carbonate solution and 2 cc. of diazotised sulphanilic acid solution, prepared according to Koessler and Hanke, in the test cylinder, x cc. of the standard carnosine nitrate solution was added from a burette. 8–10 minutes are sufficient to develop the maximum colour. This solution was then placed on one side of the colorimeter and the scale set at 10 mm. The CR. MO solution under preparation was then compared with it.

Different amounts of carnosine nitrate were treated as above and matched against the indicator solution. The results obtained were as follows:

Carnosine nitrate in test cylinders		Reading of standard indicator	Theoretical reading of standard indicator
x cc.	mg.	mm.	mm.
0.3	0.03	9.6	9.6
0.4	0.04	13.0	12.7
0.5	0.05	16.5	16.0
0.6	0.06	21.8	19.2

No sharp linear relationship between the amount of carnosine and the reading of standard indicator was therefore found but the proportionality was nearer than in Clifford's experiments. From these figures which were consistently obtained the amounts of carnosine nitrate corresponding to any depth of the standard indicator between 9.6 and 21.8 can be obtained by calculation. As the molecular formula of carnosine nitrate is $C_9H_{14}O_3N_4HNO_3$, the amounts of carnosine will be obtained by multiplying the amounts of carnosine nitrate by 0.78286.

Determination of Carnosine in certain leg muscles of the Cat.

Results obtained from ten cats by the method described are shown in the following table:

Table I.

Cat No.	Gastro- cnemius	Quadriceps	Semi- membranus Carnosine %	Triceps	Solens	Tensor
1	1.293	0.955	1.799	0.845	—	1.299
2	1.153	0.889	1.441	0.766	0.352	—
3	1.028	1.006	—	0.706	—	—
4	1.012	—	—	0.727	0.341	—
5	0.924	0.843	1.532	0.686	—	—
6	0.905	0.687	1.221	0.574	0.483	—
7	0.775	—	—	0.753	—	—
8	0.747	—	1.578	0.669	—	—
9	0.697	0.550	—	0.602	—	—
10	—	—	1.086	0.525	—	—

As the above table shows, markedly different amounts of carnosine are present in different muscles. The semimembranus contains the largest amounts

and the solens the smallest. These differences are consistent, in spite of the fact that the absolute amount of the base in any one muscle is seen to differ considerably when one animal is compared with another. Clifford did not find this wide variation in different animals. In regard to this point further investigation is therefore required. In favour of the reliability of the method as used by myself (at least when comparisons are in question), I would point to the close agreement between the amounts of carnosine found in the experiments now to be described. In these the muscles of opposite sides were compared in the same animal.

As stated above, the experiments were done in an endeavour to decide whether the phenomena involved in Tonus produce any change in the concentration of nitrogenous metabolites other than creatine. The particular experiments described in the present paper deal with a comparison between normal muscles and those in the condition of decerebrate rigidity. As a considerable difference exists in the amounts of carnosine in the same muscle in different cats (Table I), it was necessary to compare the amounts of the base on opposite sides of the same animal. On one side the occurrence of rigidity was prevented by cutting the motor nerve.

As a preliminary, a comparison was made of the muscles of normal cats on opposite sides (Table II). Next, also in normal cats, the effect of cutting the motor nerves on one side was investigated, estimations being made four hours after section of the nerves (Table III). Finally, in other animals, the nerves of one side were cut and decerebration then performed. Estimations were then made after the rigidity had existed for four hours (Table IV).

It is a well-known fact that the decerebrate rigidity can be seen in gastrocnemius, quadriceps and triceps. The estimation was made with these three muscles. The next three tables show the results respectively. Decerebration was carried out by Dr K. Uemo.

Table II.

Cat No.	Side	Gastrocnemius	Quadriceps	Triceps
1	Left	1.030	1.012	0.714
	Right	1.025	1.001	0.699
	Difference	0.005	0.011	0.015
2	Left	0.684	0.546	0.596
	Right	0.697	0.550	0.603
	Difference	-0.013	-0.004	-0.007
3	Left	0.769	0.696 0.533	0.753
	Right	0.775	0.690 0.525	0.740
	Difference	-0.006	0.006 0.008	0.013

Table III.

Cat No.	Side	Gastrocnemius	Quadriceps	Triceps
1	Left (nerve cut)	—	0.736	0.666
	Right (normal)	—	0.749	0.671
	Difference	—	-0.013	-0.005
2	Left (nerve cut)	—	0.832	0.667
	Right (normal)	0.924	0.843	0.689
	Difference	—	-0.011	-0.019 (-0.022)
3	Left (nerve cut)	—	0.683	0.579
	Right (normal)	0.905	0.687	0.574
	Difference	—	-0.004	0.005

Table IV.

Cat No.	Side	Gastrocnemius	Quadriceps	Triceps
1	Dec. rigidity	0.850	0.554	0.517
	Nerve cut	0.843	0.555	0.520
	Difference	0.007	-0.001	-0.003
2	Dec. rigidity	1.101	0.797	0.677
	Nerve cut	1.105	0.795	0.669
	Difference	-0.004	0.002	0.008
3	Dec. rigidity	1.300	0.961	0.849
	Nerve cut	1.296	0.950	0.840
	Difference	0.004	0.011	0.009

It will be seen on examination of the above figures that neither cutting the motor nerve nor the induction of decerebrate rigidity has any effect upon the carnosine in the muscles.

SUMMARY.

Using a colorimetric method based upon that of Koessler and Hanke, as modified by Clifford, estimations of carnosine have been made in the muscles of the cat.

The method gave highly consistent results when applied to corresponding muscles in the opposite limbs of the same animal.

The carnosine content of any one type of muscle varies considerably in different cats. Much wider differences, however, are to be observed in different muscles of the same animal, and a high or low concentration respectively would seem to be characteristic of individual muscles.

Neither section of its motor nerves nor the condition of decerebrate rigidity produces any effect upon the concentration of carnosine in muscle.

I wish to express my grateful thanks to Professor F. Gowland Hopkins for the interest he has taken in this work.

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