THE EFFECT OF WORK ON THE CREATINE CONTENT OF MUSCLE

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(Received September 30th, 1909)

PREVIOUS WORK

The question—Does work influence the amount of creatine in muscle, and lead to a change in the output of creatinine in the urine, has long been a debated one. Much of the older work on this point is of little value owing to the defective methods then available for the estimation of creatine and creatinine. The papers of Weber (1) and van Hoogenhuyze and Verploegh (2) give good accounts of these earlier investigations.

Of the older workers, Liebig (3) found an increase of creatine in muscle after work; as did also Sarokow (4), Szelkow (5), and Monari (6). Nawrocki (7), and Voit (8) thought that there was a slight decrease. Of modern workers Weber (*loc. cit.*), using Folin's method, found that work caused a slight decrease in the creatine content of muscle. He also found that traces of creatine and creatinine could be demonstrated in the Ringer's fluid with which, using the method of Langendorff, he had perfused an isolated heart; while Mellanby (9), also using Folin's method, obtained results from which he concluded that work had no influence on the creatine content of muscle.

As regards the effect of work on the excretion of creatinine in the urine there is also a difference of opinion. Meissner (10), employing very defective methods, found an increase of creatinine in the urine on the day of exercise, followed by a decrease on the succeeding day. Grocco (11), and Moitessier (12) also found an increased output on the day of exercise. Gregor (13) concluded from personal experiment, using a creatinine free diet, that exercise increased the output of creatinine in the urine. Voit (*loc. cit.*), Hoffmann (14), Oddi and Tarulli (15), using the older methods found no increase. Van Hoogenhuyze and Verploegh (*loc. cit.*), using Folin's method, found that, with an ample diet—creatinine free, work left the excretion of creatinine unaffected, but that when the diet was defective, as during the complete fast of their subject, La Tosca, exercise resulted in a slight increase of creatinine in the urine. Weber

(*loc. cit.*) also came to the conclusion that, if work were performed during a fast, a rise in the output of the creatinine followed. Shaffer (16) agreed with the results of van Hoogenhuyze and Verploegh. One of us (E. P. C.), although not investigating this particular point, working in conjunction with Drs. Kennaway and Leathes (17), was unable to detect any definite rise in the output of creatinine even after severe work under very different conditions, in all of which, however, the diet was ample. We may therefore accept that, provided the supply of food is sufficient, work does not bring about any increase of creatinine in the urine.

PRESENT INVESTIGATION

As regards the results on stimulating frog muscle we have already made a communication (18), in which we showed that there is an increase in the amount of total creatinine in stimulated isolated frog muscle (ordinary nerve muscle preparation), whereas, when the circulation is left intact as in the decerebrated frog, there is always a slight decrease in the amount of the total creatinine present.

The following table gives the results with frog muscle:—

TABLE I

SERIES A. (Isolated nerve muscle preparation.)			
Experiment	Per cent. of total creatinine in normal (controls)	Per cent. of total creatinine in stimulated	Difference
A	0.32	0.36	12 % increase
B	0.30	0.32	7 % „
C	0.32	0.36	13 % „
D	0.36	0.39	8 % „
SERIES B. (Muscle <i>in situ</i> , circulation intact.)			
A	0.37	0.26	15.6 % decrease
B	0.35	0.29	6.2 % „
C	0.30	0.23	9.1 % „
D	0.32	0.24	7.7 % „

We have now carried out a series of experiments on rabbit muscle under different conditions, and have obtained constant results.

The methods we employed were as follows:—The rabbit was deeply anaesthetised with ether and was kept under the influence of the anaesthetic till the end of the experiment, when it was destroyed. The extensor muscles of the right knee were exposed by means of a skin incision and removed, the amount taken being usually 10 to 15 grammes. The

bleeding points were secured and care was taken not to injure the great vessels of the thigh. All visible fatty and fibrous tissue, as well as blood, was rapidly removed from the excised mass, which was then weighed, transferred to a small mortar, minced fine by means of a pair of sharp scissors, then rubbed up with finely powdered glass, water being gradually added until a fine suspension was obtained. This mixture was next carefully transferred to an Erlenmeyer flask, filled up to 150 c.c. with distilled water, some chloroform and thymol solution added, thoroughly shaken up, and then placed in a hot water oven at 50° C., where it was left, being repeatedly shaken, for eight to ten hours. At the end of this period the flask, after faintly acidifying its contents with acetic acid, was put on the steam bath, or boiling water bath, for thirty minutes and then filtered. The solid residue was extracted with boiling water (as a rule by boiling the residue with water in a porcelain basin) five to seven times and filtered through the original filter. The united filtrates were then concentrated to 40 c.c., and the content of creatine + creatinine estimated by Folin's colorimetric method. The same procedure was carried out in the case of the stimulated extensor muscles of the left thigh, care being taken (i) that the corresponding muscles were taken for examination, and (ii) that the amount taken differed by several grammes from that taken from the right (unstimulated) side—sometimes more being taken, sometimes less—so that the readings on the colorimeter were widely apart, thus obviating to a considerable extent any possible personal bias. As an additional precaution one of the observers frequently manipulated the solutions in such a manner that the observer who took the readings was unaware which of the two solutions he was examining. The reason why definite corresponding muscles were chosen was that as the result of several experiments we carried out with different kinds of muscle (red and white) we are inclined to believe that there is some difference in their creatine content. That such might be the case is suggested by such work as that of Bonhöffer (19) and of Paukul (20), who showed that in muscle structural differences might be associated with functional. So far we have not performed a sufficient number of experiments on this point to be able to draw definite conclusions.

As regards our method of stimulation, immediately after the removal of the extensor muscles of the right side those of the left were transfixed by a pair of electrodes, one of which passed through the muscles close to the patella and the other near their origin. Through these electrodes the muscles were stimulated with rapid alternating faradic shocks. A

Berne coil was used, the primary current being about 5 volts and the secondary coil was placed between the 2,000 and 4,000 unit marks. The muscles were stimulated over a period of thirty to forty-five minutes, but the secondary currents were not allowed to pass continuously through them, being short-circuited for five seconds in every ten seconds.

We carried out control experiments to determine the accuracy of our chemical methods, and found that, for small amounts of muscle, the average difference between the extensors of the two sides, neither having been stimulated, was a little over 1 per cent. of the total creatinine, and for larger amounts (10 to 15 grammes) was about 2·3 per cent.

The general result of the whole series of experiments has been that, with the circulation intact, stimulation of the muscles brings about a constant, although small, decrease in the amount of total creatinine (i.e., creatine + creatinine) extracted from the stimulated muscle.

Experiment II.—Rabbit, weight 1,550 grams. 12 grams muscle removed before stimulation (stimulation 30 minutes) and 11 grams after. Total creatinine before stimulation, 43·42 milligrams = 0·361 %, and after, 30·84 milligrams = 0·280 %, i.e. a decrease of 22·4 % as a result of stimulation.

Experiment IV.—Rabbit, weight 1,850 grams. 7·5 grams muscle removed before and 17 grams after 40 minutes' stimulation. During the period of anaesthesia a free supply of pure oxygen was given. Total creatinine before stimulation 28·8 milligrams = 0·334 %, after stimulation 54·6 milligrams = 0·321 %, i.e. a decrease of 4·3 % as a result of stimulation.

Experiment V.—Rabbit, weight 1,450 grams. 9·2 grams muscle removed before and 16 grams after 30 minutes' stimulation. Total creatinine obtained before, 37·84 milligrams = 0·411 %, and 52·4 milligrams after = 0·327 %, i.e. a decrease of 20·4 %.

Experiment VI.—Rabbit, weight 2,200 grams. 10 grams muscle removed before and 12·5 grams after 30 minutes' stimulation. A free supply of pure oxygen was allowed during the period of anaesthesia. Total creatinine before stimulation 30·60 milligrams = 0·306 %, and 37·68 milligrams after = 0·301 %, i.e. a decrease of 1·6 %.

Experiment IX.—Rabbit, weight 2,250 grams. 9·1 grams muscle removed before and 14·2 grams after 35 minutes' stimulation. Total creatinine in muscle, before stimulation 39·04 milligrams = 0·429 %, and 51·42 milligrams after = 0·361 %, i.e. a decrease of 15·8 %.

Experiment X.—Rabbit, weight 1,900 grams. 11·5 grams muscle removed before, and 14·8 grams after 35 minutes' stimulation. Total creatinine in muscle, before 43·24 milligrams = 0·375 %, and 50 milligrams after = 0·337 %, i.e. a decrease of 10·1 %.

As an example of two control experiments done, the following may be given, No. 7 with our maximum error, and No. 12 with our minimum.

Experiment VII.—Rabbit. 15 grams of muscle removed from right, and 13·1 grams from left thigh. Total creatinine, in muscle from right side 53·08 milligrams = 0·354 %, and from left side 48·0 milligrams = 0·366 %, i.e. a difference of 3·3 %.

Experiment XII.—Rabbit. 8 grams of muscle removed from right thigh and 11 grams from left. Total creatinine, in muscle of right side 36·93 milligrams = 0·461 %, and from left side 50·31 milligrams = 0·457 %, i.e. a difference of 0·86 %.

If Mellanby's figures be examined, although he draws the conclusion from them that work is without influence on the creatine content of muscle, it will be noted (*loc. cit.* pp. 459-460) that his frog's muscle stimulated when isolated always show a slight increase in amount of total creatinine present, perhaps somewhat smaller than ours, and, in the two experiments on rabbits, which he did with their circulation intact, there is in both cases a slight decrease.

As already mentioned, van Hoogenhuyze and Verploegh have come to the conclusion, in which they are supported by other investigators, that work does not increase the output of creatinine in the urine when the diet is sufficient.

It was thought that the state of the nutrition of the animal, or, perhaps, variations in the carbohydrate reserve, might influence the total creatinine content of worked muscle. The experiments given above may be divided into two series, one in which the animals were kept on a very low cabbage diet for four or five days preceding the experiment, and the other in which the animals were allowed to feed freely on bran, turnip, and carrot for the same length of time. In every case there was a decrease in total creatinine, but the decrease was somewhat greater in the badly fed than in the well fed animals.

TABLE II.

SERIES A. Badly fed				
Experiment	Total creatinine in milligrams	Per cent. present in muscle	Decrease per cent.	Mean decrease per cent.
2. { B(efore) A(fter)	43.42 30.84	0.361 0.280	22.4	
5. { B. A	37.84 52.40	0.411 0.327	20.4	
10. { B A.	43.24 50.00	0.375 0.337	10.1	
			52.9	17.63
SERIES B. Well fed				
4. { B. A.	28.8 54.6	0.334 0.321	4.3	
6. { B. A.	30.60 37.68	0.306 0.301	1.6	
9. { B. A.	39.04 51.24	0.429 0.361	15.8	
			21.7	7.23

Other experiments with frogs were carried out along these same lines, previous to the rabbit experiments. In these the frogs were given large quantities of glucose for two days before stimulation, but the results which we obtained by this method were not very concordant.

Another point which appeared in the course of our work was the constant gain in fluid which occurred in the muscle as a result of stimulation. We found that, in the muscles of both frogs and rabbits, the average gain after thirty to forty minutes' stimulation was about 2 per cent. We discovered subsequently that this figure was almost identical with that given by Ranke (21). In our experiments the fresh muscle was weighed as soon as possible after removal, then dried at 100° C. till constant weight was obtained.

In every experiment, although our method is not calculated to elucidate the question, we examined the muscle extract for preformed(?) creatinine, but found that the amounts present varied very largely in quantity, the variation being also found in the control experiments. We are inclined to agree with Mellanby that muscle contains no preformed creatinine, or at most mere traces of this substance. The variation in the amounts found in our experiments were due in all probability to a partial conversion of creatine to creatinine during the process of extraction. There was, however, one interesting fact which may be mentioned in this connection, and that was that the percentage amount of preformed (?) creatinine present in the above experiments with rabbit muscle showed, with the exception of one experiment, a decrease when there was a decrease of total creatinine.

The expenses of this research have been defrayed by a grant from the Carnegie Trust.

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