THE CREATIN CONTENT OF MUSCLE IN MALIGNANT DISEASE AND OTHER PATHOLOGICAL CONDITIONS

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Since Folin¹ in 1904 published his adaptation of Jaffé's picric acid test to the quantitative estimation of creatin and creatinin, much work* has been done on the circumstances which govern the appearance of creatin in the urine; but, so far as I am aware, no figures have yet been published showing the alterations that occur in the amount of creatin in the muscles of man in various conditions as estimated by this method. The present communication shews the results of some work undertaken to fill this gap in our knowledge.

The method adopted was that given by Mellanby², and is as follows:—At the time the body was opened for the post-mortem examination, part of the pectoral muscles, usually about 40 grams, was removed and freed from fat and connective tissue. It was then finely divided with scissors, and ground up in a mortar with 97 per cent. alcohol. After standing for twenty-four hours the alcohol was strained off through muslin, and the residue extracted four times with distilled water, the last drops of the extract being squeezed out in a meat-press. Control experiments showed that all the creatin is extracted by this process. The extracts were then added together and evaporated to dryness on a water bath: the dry residue was then extracted with 75 per cent. alcohol, filtered, and made up to a definite volume, usually 200 c.c. An aliquot part of this was then evaporated to dryness and extracted with a measured amount of distilled water. Finally 20 c.c. of the extract was taken and heated on a water bath for five hours with a 5 c.c. of normal HCl, the volume being kept constant. After cooling, the acid was exactly neutralised with normal KOH solution; and the creatin, which by this process is entirely converted into creatinin, estimated by Folin's method. The figure arrived at expresses the amount of creatinin present, and the creatin figures shewn in the tables are obtained by multiplying the creatinin figures by 1.16 (Folin).

* For references to the literature, see Mendel and Rose. *Journ. of Biol. Chem.*, Vol. X, p. 213, 1911.

Mellanby and others* have shown that creatin alone is present in muscle, to the exclusion of creatinin. In several cases the mixed alcoholic and watery extracts, which had undergone no treatment capable of converting creatin into creatinin, were tested for the presence of the latter body, which was however always absent.

At Guy's the bodies awaiting post-mortem examination are kept in a cold chamber at a temperature of about 40° F. In view of the possibility that during the period of waiting some alteration might be produced in the creatin content of the muscles, control experiments were undertaken with rabbit's muscle. In two cases rabbits were killed with chloroform, and the creatin estimated at once in the shoulder-girdle muscles, thigh muscles, and rectus abdominis (Rabbit A), or erector spinae (Rabbit B) of one side. The rabbit was then wrapped in a cloth and placed in the cold chamber for twenty-four hours. At the end of that time the creatin was estimated in the corresponding muscles of the opposite side of the body. The results are shewn in the following table:—

TABLE I.—Rabbit A, male, 2,500 grams

Creatin as percentage of fresh muscle.

				At once	After 24 hours	Difference
Thigh muscles	...	...	...	0.296	0.279	5.7%
Shoulder girdle	...	...	...	0.306	0.290	5.5%
Rectus abdominis	...	...	...	0.253	0.217	14.2%
Average	...	...	...	0.285	0.262	8.1%

Rabbit B, male, 1,270 grams.

Thigh muscles	...	...	...	0.384	0.349	9.1%
Shoulder girdle	...	...	...	0.305	0.204	33.2%
Erector spinea	...	...	...	0.364	0.352	3.3%
Averages	...	...	...	0.351	0.302	14.0%

The table indicates that a stay of twenty-four hours in the cold room lessens the amount of creatin in the muscles. The number of hours elapsing between death and removal of the muscle from the body has therefore been noted in all cases. Further, there appears to be a difference in the creatin content of muscles from different parts of the same body; in all cases, therefore, the same muscle (the pectoral) has been used.

* The first observers to make this statement were Toppelius and Pommerehne. *Archiv. de Pharm.*, Vol. CCXXXIV, p. 380, 1896. Their work has been confirmed by Wörner, *Zeits. f. Physiol. Chemie*, XXVII, p. 1, 1899, and by Grindley and Woods, *Journ. of Biol. Chem.*, II, p. 309, 1906-7.

The Creatin Content of Normal Muscle

In order to arrive at the creatin content of normal muscle, the cases selected were all young adults who had met with an accident, and were either brought in dead to the hospital or died shortly after admission, and in whom no abnormality, apart from their injuries, could be found. As permission has to be obtained from the coroner in cases of accident before the post-mortem can be made, the time elapsing between death and the post-mortem examination is considerably larger than in any of the other groups of cases. As will be seen, the results are remarkably uniform, but rather lower than those given by Hofmann³ (0·282—0·316 per cent.), who worked with Neubauer's method. This may be partly due to the destruction of creatin caused by the prolonged stay in the cold room, and also in part to the imperfections of the method employed by Hofmann. If we estimate the loss of creatin during the stay in the cold room at 10 per cent., we arrive at 0·300 per cent. as the creatin content of the muscles of a healthy young adult.

TABLE II.—Normal Adults

	Sex	Age	Injury	Hours after death	Creatin as percentage of fresh muscle	
1.	Male	...	27	Multiple fractures	32	0·257
2.	Male	...	31	Fractured spine	30	0·290
3.	Male	...	27	Ruptured lung and spleen, fractured ribs	39	0·271
4.	Male	...	22	Multiple fractures	36	0·280
5.	Male	...	35	Fractured ribs	46	0·251
6.	Male	...	38	Fractured tibia and fibula	20	0·268
Averages			...	30	34	0·270

THE CREATIN CONTENT IN PATHOLOGICAL CONDITIONS

The pathological conditions may be separated into the following groups:—

1. Cases of carcinoma and sarcoma.
2. Cases of acute disease of short duration.
3. Cases of subacute and chronic disease:—
 - (a) Accompanied by marked loss of weight.
 - (b) Not accompanied by marked loss of weight.

1. *Cases of Carcinoma and Sarcoma*

It has been shown by Mellanby in the paper already quoted that patients suffering from malignant disease frequently excrete creatin in their urine, and Shaffer⁴ has never failed to find it under these conditions. This excretion is specially marked in patients suffering from growths in the liver. Indeed in Mellanby's cases the amount of creatin in the urine in such cases was greater than the amount of creatinin, and the same was true on many occasions of a case of secondary carcinoma of the liver that was under my own observation for some time. The cases of carcinoma and sarcoma have, therefore, been grouped together in Table III.

TABLE III.—Cases of Sarcoma and Carcinoma

Sex	Age	Disease	Hours after death	Creatin as percentage of fresh muscle
1. Female ...	62	Carcinoma of colon and liver	12	0.186
2. Male ...	71	Carcinoma of cheek abscess of lung	28	0.201
3. Female ...	38	Carcinoma of pylorus	25	0.199
4. Female ...	37	Sarcoma of uterus	16	0.232
5. Male ...	67	Carcinoma of stomach	24	0.315
6. Female ...	68	Carcinoma of rectum, deposits in liver and lung	27	0.167
7. Male ...	50	Carcinoma of gall bladder and liver	27	0.212
8. Female ...	58	Carcinoma of pancreas	21	0.228
9. Male ...	65	Carcinoma of hepatic duct, jaundice	3	0.226
10. Male ...	52	Carcinoma of rectum	8	0.210
Averages ...	57		19	0.218

In spite of the fact that the average interval between death and the post-mortem examination is 44 per cent. less than in the normal cases, there is a decrease in the average creatin content of the muscles of 19 per cent., and this decrease is shewn by all the ten cases, with the exception of Case 5, where the creatin is for some undiscovered reason abnormally high. It will be noticed that the amount of creatin is lowest of all in Cases 1 and 6, where there were large masses of growth on the liver; in Case 7, where the content of creatin is somewhat larger, the growths on the liver were both smaller and fewer. The appearance of creatin in the urine of patients suffering from malignant disease is apparently due to a larger muscle breakdown, as suggested by Mellanby,*

* In support of this Shaffer's results may be quoted. This observer found the largest amount of creatin in the urine in the case of puerperal involution of the uterus, where muscle breakdown is at its highest.

following Benedict and others;⁵ and this muscle breakdown is most marked in cases of carcinoma of the liver, that is to say, in cases marked clinically by excessive loss of body weight.*

This excessive muscle breakdown may not, however, be the sole cause of the low creatin content of the muscles in this class of case. Mellanby has shewn that there are reasons for supposing that muscle creatin is formed in the liver, and that one of the functions of that organ is to keep the amount of creatin in the muscles up to a certain standard. It is, therefore, quite possible that the low creatin content is not due merely to excess of muscle breakdown, but is also due in part to failure on the part of the liver to make good the usual loss. In both Cases 1 and 6 the amount of liver substance remaining was obviously much less than normal, and it is a point of some interest in this connection that the lowest creatin content in Table V, which consists of cases where no wasting was observed clinically, is found in Case 6, where much of the parenchyma of the liver was replaced by connective tissue: diminished creatin production as well as increased creatin loss may therefore both be factors in the causation of the low creatin content of the muscles in cases of hepatic disease.

2. Cases of Acute Disease of Short Duration

The cases in the following table showed no muscular wasting, and the creatin content is that of the normal adult, and requires no comment.

TABLE IV.—Cases of Acute Disease

Sex	Age	Disease	Hours after death	Creatin as percentage of fresh muscle
1. Male	10	Intus-susception. Operation—general peritonitis	11	0.275
2. Male	65	Acute obstruction by band. Operation	22	0.285
3. Female	7	Intus-susception. Operation—general peritonitis	19	0.280
4. Male	59	Appendicitis. Operation—general peritonitis	15	0.328
5. Male	21	Lateral sinus thrombosis, septicaemia	12	0.215
6. Male	59	Acute ptomaine poisoning	13	0.240
Average	37		15	0.270

* A possible factor in the origin of the large amount of creatin found in the urine in cases of carcinoma of the liver is the production of creatin by the new growth itself. That this factor is comparatively unimportant is shown by the fact that in one case the percentage of creatin in a mass of new growth taken from the liver was only 0.014, while in two other cases the amount could not be measured accurately, but was even less than this. Even supposing the growth to weigh 1,000 grams, this only represents 0.14 grams of creatin, while the total creatin excretion per diem in Mellanby's cases averaged 1.18 grams. Cf. Saiki, *J. Biol. Chem.*, p. 23, VII, 1909-10.

3. *Cases of Subacute and Chronic Disease*

(a) Not accompanied by marked loss of body weight.

(b) Accompanied by marked loss of body weight.

TABLE V.—Subacute and Chronic Disease, without marked loss of weight

Sex	Age	Disease	Hours after death	Creatin as percentage of fresh muscle
1. Male ...	48	Chronic interstitial nephritis, pericarditis	48	0.268
2. Male ...	36	Aortic disease	12	0.232
3. Male ...	56	Fibroid lung	14	0.278
4. Male ...	59	Myocardial degeneration	27	0.224
5. Female ...	76	Diabetes	50	0.257
6. Female ...	58	Gall stones, cirrhosis	9	0.202
Averages ...	55		27	0.244

TABLE VI.—Subacute and Chronic Disease, with marked loss of weight

Sex	Age	Disease	Hours after death	Creatin as percentage of fresh muscle
1. Male ...	10	Appendicitis, subdiaphragmatic abscess	6	0.259
2. Female ...	13	Phthisis	9	0.184
3. Female ...	17	Infective endocarditis, pericarditis	20	0.184
4. Male ...	31	Laceration of liver and kidney, general peritonitis	22	0.219
5. Female ...	15	Chronic peritonitis, intestinal obstruction	17	0.203
6. Male ...	32	Phthisis	10	0.266
7. Male ...	9	Adherent pericardium	23	0.147
8. Male ...	58	Hodgkins' disease, a few nodules in liver	24	0.260
Averages ...	23		16	0.215

The cases in Table V showed no obvious signs of loss of weight during life, and no mention is made of loss of weight in the reports. The statement, however, rests usually on the patient's own impression, and in no case were the actual weights given. There may, therefore, easily have been some slight unnoticed loss, which would account for the fact that the creatin content is somewhat below the normal for healthy adults. All the cases in Table VI were accompanied by loss of flesh so marked as to attract the patient's own attention, and here the average creatin content is the same as that found in cases of malignant disease.

SUMMARY

1. The average creatin content of the muscles of a healthy adult is 0·300 per cent. of the fresh weight.

2. The creatin content is reduced in cases of malignant disease, especially in cases of growth in the liver, and there is reason to suppose that in these latter cases the low content is due to diminished production as well as to increased loss.

3. The content is not reduced in cases of rapidly fatal acute disease, but is lowered in cases of subacute and chronic disease, where there has been marked loss of body weight.

REFERENCES

1. Folin, *Zeitschrift f. Physiol. Chemie*, XLI, p. 223, 1904.
2. E. Mellanby, *Journ. of Physiol.*, XXXVI, p. 447, 1908.
3. Hofmann (quoted by Voit), *Zeitschrift f. Biologie*, IV, p. 82, 1868.
4. Shaffer, *American Journ. of Physiol.*, XXIII, p. 1, 1908.
5. Benedict, *Carnegie Institute of Washington*, Publication No. 77, 1907.
Benedict and Myers, *Amer. Journ. of Physiol.*, Vol. XVIII, p. 406, 1907.