CCXXIII. THE INFLUENCE OF THE ADMINISTRATION OF ARGININE AND HISTIDINE ON THE EXCRETION OF CREATININE.

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THE numerous attempts to demonstrate the origin of muscle-creatine or urinary creatinine from arginine or histidine have been almost entirely negative in result or open to serious criticism. The fact that both arginine and histidine are subject to destruction by the liver has no doubt militated against any positive result in the majority of cases, especially when small amounts of the amino-acids have been administered *per os*.

In regard to histidine more definite evidence has recently been presented by Abderhalden and Buadze [1929; 1930], who have shown that feeding large amounts of histidine or haemoglobin led to significant increases in creatinine excretion.

Reviews of the literature dealing with the effect of administering arginine, histidine, cystine and other substances are given by Hunter [1928], Hyde and Rose [1929] and Abderhalden and Buadze [1929]. In a recent paper Beard and Barnes [1931] reported the effects of administration of protein, amino-acids and related substances on muscle-creatine and urinary creatinine in adult rats and also the effects of these substances on creatinine elimination in man. A large number of amino-acids, including histidine, arginine, glycine, glutamic acid, tyrosine and cystine, the proteins edestin and caseinogen, and finally choline and glycocyanine were found to increase muscle-creatine. Negative results were obtained with sarcosine, nucleic acid, betaine and taurine. All the amino-acids and proteins studied increased the elimination of creatinine.

These authors believe that their results cannot be ascribed to the effect of specific dynamic action and conclude that the creatine formation and creatinine excretion were due either to feeding large quantities of proteins and amino-acids or to the possibility that these substances stimulated creatine-creatinine metabolism in some other way than by specific dynamic action. They favour the former view but taking into account the large number and variety of substances giving positive results a more likely explanation is one based on the assumption of a generalised stimulation of creatine-creatinine metabolism. However, several investigators [Lewis, Dunn and Doisy, 1918;

Zwarenstein, 1928; Christman and Mosier, 1929] have shown that administration of several amino-acids, including glycine which gave the maximum effect in Beard and Barnes' experiments, had no effect on creatinine excretion. A satisfactory explanation of the above results is difficult and the findings of Beard and Barnes are in obvious need of re-examination.

In the work described below attention has been focused largely on the effects of arginine and histidine but some of the experiments of Beard and Barnes with other amino-acids have been repeated. During the course of research on other lines in this laboratory it was found that rabbits were very suitable animals for investigations on creatine-creatinine metabolism. The daily output of creatinine is constant within comparatively narrow limits except for slight seasonal variations and is furthermore independent of caging or of the usual diet of carrots, cabbages and bran. For short period experiments such as those described below the normal daily creatinine excretion remains remarkably constant.

EXPERIMENTAL.

The experiments were carried out on adult male rabbits. Details in regard to caging, feeding and collection of urine have been described elsewhere [Schrire and Zwarenstein, 1932]. Creatine and creatinine were estimated by Folin's method. Eight animals were used in all and before the effects of the various substances were investigated the daily creatinine excretion was estimated for periods of 4 or 5 days over several weeks and thus a normal pre-period consisting of about 18 daily creatinine figures was obtained. On the day preceding administration of the substance creatinine was again determined thus checking any large deviation from the normal.

The histidine hydrochloride, arginine, glycine and alanine were dissolved in Locke's solution (30-35 cc.) and the solutions of the first two were neutralised with dilute NaOH or HCl before injection. Cystine, tyrosine, glutamic acid and glycocyamine were fed mixed with bran. Only in the case of cystine did the animals fail to ingest all the mixture, so that the amounts for cystine in Table II are actually rather less than stated. In some experiments the animals received a single injection, in the majority of experiments they were injected or fed once a day on three consecutive days. Four animals were usually put into separate metabolism cages at the same time. One of these always received Locke's solution only or bran and served as a control. Every animal in its turn served as a normal control in the various experiments. In all cases the diet consisted of cabbages and carrots. The controls for the feeding experiments were given bran in addition. For the sake of convenience the data in the following Tables are arranged in a slightly different sequence from that in which they were actually obtained. Injection of 35 cc. of Locke's solution daily for 3 days had no effect on the excretion of creatinine. Similarly, the addition of bran to the usual ration had no effect. Small quantities (10 to 50 mg.) of the different amino-acids were added to 10 cc. portions of a filtered

urine, and the creatinine was estimated and compared with the value obtained from the same urine without the addition of the amino-acid. The readings were identical in all cases except on addition of alanine or cystine and for these two substances the differences were not significant.

The figures in the Tables below unless otherwise stated refer to mg. creatinine excreted in 24 hours. The figures in brackets refer to the amount of the substance in g. fed or injected.

Table I.

Rabbit No. Weight (kg.)	...	I 2.46	II 2.52	III 2.18	IV 2.03
<i>A. Pre-period</i>					
No. of 24 hour periods		18 96 ± 2	16 98 ± 5	18 90 ± 6	17 92 ± 5
<i>B. Arginine</i>					
Preceding day		100	101	90	95
Injection period (1 day)		109 (5)	136 (5)	106 (5)	89 (2.5)
		103	101	103	99
		107	93	93	103
		102	110	80	99
Preceding day		—	—	—	91
Injection periods (3 days)		—	—	—	91 (2.5)
		—	—	—	114 (3.0)
		—	—	—	114 (5.0)
<i>C. Histidine</i>					
Preceding day		99	106	84	92
Injection period (1 day)		94 (3)	138 (5)	104 (3.5)	109 (3.5)
		100	126	96	96
		110	109	85	80
		103	113	—	—
Preceding day		104	—	93	—
Injection periods (3 days)		125 (2.5)	—	93 (2.5)	—
		96 (3.5)	—	96 (3.0)	—
		117 (4.0)	—	120 (5.0)	—

Table II.

Rabbit No. Weight (kg.)	...	III 2.18	IV 2.03	V 1.80	VI 1.77
<i>A. Pre-period</i>					
No. of 24 hour periods		18 90 ± 6	18 92 ± 5	18 72 ± 4	18 72 ± 3
<i>B. Glycine</i>					
Preceding day		92	90	75	70
Injection periods (3 days)		91 (4.5)	85 (4.5)	65 (5)	70 (5)
		96 (3.5)	96 (3.5)	78 (5)	73 (5)
		91 (3.5)	92 (3.5)	70 (3)	69 (3)
<i>C. Alanine</i>					
Preceding day		90	93	72	80
Injection periods (3 days)		92 (4.5)	87 (4.5)	67 (4)	70 (4)
		80 (3.5)	93 (3.5)	72 (4)	75 (4)
		90 (3.5)	80 (3.5)	65 (3)	72 (3)
<i>D. Cystine</i>					
Preceding day		82	90	—	80
Feeding periods (2-3 days)		83 (5)	90 (3.5)	—	70 (3.5)
		80 (1)	90 (4)	—	69 (4)
		86 (4)	—	—	65 (3.5)

Table III.

Rabbit No. Weight (kg.)	...	III 2.18	IV 2.03	VII 1.80	VIII 1.99
A. <i>Pre-period</i>					
No. of 24 hour periods		18 90 ± 6	18 92 ± 5	18 73 ± 5	18 76 ± 6
B. <i>Tyrosine</i>					
Preceding day		85	91	71	73
Feeding periods (2-3 days)		90 (5) 93 (5) 85 (3)	89 (4.5) 94 (10) —	76 (5) 70 (5) 73 (3)	70 (5) 75 (5) 80 (3)
<i>Glutamic acid</i>					
Preceding day		96	92	69	73
Feeding periods (2-3 days)		90 (5.5) 98 (3.0) 85 (4.0)	89 (7) 93 (5.5) 87	70 (5.5) 69 (3.0) 69 (4.0)	73 (7.0) 77 (5.5) 76
<i>Glycocyamine</i>					
	Rabbit No.	II		III	
		Preformed creatinine	Creatine	Preformed creatinine	Creatine
Preceding day		80	0	84	0
Feeding periods (2-3 days)		92 95 90 94	39 (3) 47 (4) 107 (3) 161	91 68 — —	179 (3) 269 (3) — —

Variable amounts of creatine (0-36 mg.) were found in the urine after injection of arginine and histidine, but similar amounts were excreted during the normal pre-periods and also after injection of Locke's solution. Consequently the occurrence of creatine after injection of the amino-acids cannot be regarded as significant.

DISCUSSION.

The results in Table I show that the injection of arginine and histidine caused increases in urinary creatinine of the order of 10-40 %. The effects were definite in all the animals injected and although the reaction to arginine and histidine in any one animal was very similar, individual variations occurred. Single day injections into animal II gave increases of about 40 %, in I the increases were less (about 14 %). Injections on successive days caused these increases to persist; in some cases further rises occurred. These effects are in striking contrast to those obtained with other amino-acids. In Tables II and III it is seen that none of the amino-acids, glycine, alanine, cystine, tyrosine and glutamic acid, had any effect on the excretion of creatinine or creatine, whether administered subcutaneously or *per os*. The results in Tables II and III indicate further that the increases obtained with arginine and histidine could not have been due to specific dynamic action.

The large increases in creatine excretion as a result of feeding glycocyamine confirm the results of previous investigators. The preformed creatinine, except for a drop on the second day in animal III, remained unaffected.

It is highly significant that with arginine and histidine which have struc-

tural affinities to creatine and creatinine there are prompt and definite increases in creatinine, while with all the other amino-acids, which have no such direct relationship, there is no effect. Glycocyamine increases creatine excretion but has no effect on creatinine. An analysis of Table I indicates that arginine and histidine are identical in their effects upon urinary creatinine. From this it may be concluded that the iminazole nucleus in histidine and the guanidine nucleus in arginine undergo transformation into creatinine (either directly or through the intermediate stage of creatine).

On the other hand when the guanidine nucleus is introduced in the form of glycocyamine it is transformed directly into creatine which is excreted as such. This difference in effect may be due to the fact that arginine and histidine were injected and glycocyamine was administered *per os*, that is, that the locus of transformation in the former case was muscle and in the latter the liver. Reviewing the evidence in regard to the site of formation of creatinine from creatine Hunter [1928] comes to the conclusion that the liver plays no part in this change and that the muscles are most probably the chief site of creatinine formation. This does not exclude the possibility of creatine formation from glycocyamine in the liver; consequently the liver probably can convert glycocyamine into creatine but cannot convert creatine into creatinine. The creatine is formed from glycocyamine rapidly and in large amounts and is excreted as such. The rapidity with which creatinine arises from arginine and histidine suggests that its formation is a direct one, but the formation of creatine as an intermediary cannot be definitely excluded. The point raised in regard to the different effects obtained with parenteral or oral administration of creatine-creatinine precursors requires further detailed investigation and may lead to an explanation of the contradictory results encountered in the literature.

The above results although not conclusive indicate that arginine and histidine are normal precursors of creatine or creatinine and suggest that only those substances with close direct chemical relationships to creatine or creatinine can be taken into account in any attempt to elucidate the still rather obscure problem of the significant biochemical processes involved in creatine and creatinine metabolism and their final interrelationships.

SUMMARY.

1. Injection of arginine and histidine into adult male rabbits gives rise to a 10–40 % increase in the elimination of urinary creatinine.
2. Feeding with glycocyamine has no effect on creatinine excretion but leads to a large output of creatine.
3. The amino-acids glycine, alanine (injected) and tyrosine, cystine, glutamic acid (ingested) have no effect on creatine or creatinine excretion.
4. It is suggested that the transformation of arginine and histidine into creatinine takes place in the muscles probably *via* creatine and that the glycocyamine-creatine change is a direct one taking place in the liver.

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