CREATINE FORMATION IN LIVER AND IN KIDNEY*

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We reported recently (1) the formation of creatine from glycocyamine by rat liver slices; and that 40 to 50 per cent more creatine was formed when methionine was added with the glycocyamine to the Ringer's solution in which the slices were immersed. Among some thirty odd amino acids, methylated amines, a methylated purine, and betaine only methionine gave this increased rate of methylation. The rate of creatine formation under these conditions is sufficient, if it is of the same order of magnitude *in vivo*, to make good the entire loss as urinary creatinine.

If the methylation of glycocyamine in the liver is important both as a mechanism and as a site of creatine formation in animals, one may expect to find it in the livers of most other animals. We accordingly examined the livers of a number of animals for their ability to carry out this reaction. At the same time the possibility of its occurring in their kidneys was investigated also.

The findings in this survey, summarized in Table I, were that glycocyamine can be methylated by the liver of every animal examined; the stimulating effect of methionine observed with rat liver was found with some but not all the other animals; the kidney appears to be unimportant (except in the pigeon) for the methylation of glycocyamine without or with methionine.

The experimental and analytical procedure was the same as was described in our previous communication, except that the experiments with frog liver and kidney were carried out at 25° instead

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of 38°. All the creatine figures given represent differences in chromogenic material in the Jaffe reagent before and after digestion with the NC bacteria of Miller and Dubos (4).

Baker and Miller (3) have reported that creatine is formed from unidentified precursors in rat liver and kidney (slices and mash). Our findings on this point are that small amounts of creatine (beyond the experimental error) were formed from pre-

TABLE I

Formation of Creatine in Liver and Kidney Slices

Temperature 38°; glucose-Ringer's solution. The creatine is given as mg. per 100 gm. of wet weight of tissue.

Animal	Creatine in liver				Creatine in kidney			
		After 4 hrs. incubation				After 4 hrs. incubation		
	Initially present	In Ringer's solution alone	With 6.25 mg. per cent glycocyamine	With 6.25 mg. per cent glycocyamine and 40 mg. per cent *dl*-methionine	Initially present	In Ringer's solution alone	With 6.25 mg. per cent glycocyamine	With 6.25 mg. per cent glycocyamine and 40 mg. per cent *dl*-methionine
Cat	12.0	12.2	21.1	23.2	1.9	2.5	2.4	2.3
Dog	2.9	2.9	3.5	10.3	17	26	26	26
Guinea pig	1.7	3.4	12.4	12.1	3.0	4.5	8.5	6.6
Frog (25°)	2.2	2.2	4.8	8.4	36	44	47	45
Pigeon	1.0	2.0	11.8	16.0	10.8	10.7	17.9	22.3
Rabbit	5.8	5.7	26.4	12.0	9.4	15.2	15.0	15.4
Rat	1.4	2.8	20.6	28.4	10.8	18.6	16.3	18.2

The initial values are lower than those given by Baker and Miller (2, 3) for rat liver and kidney. In our procedure the slices are washed in a large volume of Ringer's solution for at least 3 minutes before they are transferred to the reaction vessels or submitted to analysis. Preformed creatine undoubtedly diffuses out of the slices during this washing.

existing precursors in the liver slices of the guinea pig, pigeon, and rat. Kidney slices gave significant increases in every case except in the pigeon.

After the cell structure of rat liver and kidney was destroyed, no creatine was formed from preexisting precursors nor from glycocyamine without or with methionine. The homogenizing procedure of Potter and Elvehjem (5) was used to disrupt the cells. It would seem that the creatine formed by chopped rat liver and

kidney from preexisting precursors (Baker and Miller (3)) must be ascribed to the intact cells in the mash rather than to a liberated enzyme.

It is difficult to account for the large (absolute) amounts of creatine formed from preexisting precursors in the kidney slices in every case except in the pigeon, in view of the inability of cat, dog, rabbit, and rat kidney slices to methylate added glycocyamine, even in the presence of added methionine. The one animal which was negative in this respect—the pigeon—was the only animal whose kidney gave a positive effect with glycocyamine and a further increase with methionine.

When the Ringer's solution contained glycocyamine, there were large increases in creatine with the liver slices of every animal except the dog. In the interpretation of this negative effect in the case of the dog the positive effect observed when methionine was added with the glycocyamine must be taken into account. One possible interpretation is that the preexisting concentration of the glycocyamine-methylating agent is very low in dog liver—lower than in the livers of any of the other animals investigated.

With kidney slices the added glycocyamine was not methylated or was methylated only to an insignificant extent in every case except in the guinea pig and pigeon.

Bodansky (6) found an increase in the creatine content of rat kidney after glycocyamine was fed. Our interpretation of this increase was that it arose from temporary storage in the kidney of creatine synthesized in the liver from the glycocyamine fed (1). This interpretation is based on the finding that rat kidney slices do not methylate glycocyamine under conditions in which methylation readily occurs in liver. Bodansky concurs in this interpretation (personal communication).

Methionine accelerated the methylation of glycocyamine by liver slices of the dog, frog, pigeon, and rat. This stimulation was not observed with the cat, guinea pig, and rabbit. Two possible reasons for this failure are that the livers of these animals contained a plethora of the methylating agent, or that the relatively high concentration of the unnatural isomer in the *dl*-methionine used was inhibitory (as in the rabbit liver slices).

The pigeon was the only animal whose kidney slices were stimulated by methionine in their methylation of glycocyamine.

The pigeon is exceptional as far as creatine formation is concerned in that its kidney appears to share this function with its liver and resembles in this respect the livers rather than the kidneys of the other animals.

The negative effect of methionine with the kidney slices of all the animals tried except the pigeon is a little unexpected. Oxidative deamination is more active in kidney than in liver slices (7); and after oxidative deamination the lability of the S-methyl group is increased (8). It might have been expected therefore that the methylation of glycocyamine would be more active in kidney than in liver. This is not the case.

Our finding in this survey, that methylation of glycocyamine is an active function of the livers of all the animals examined, strengthens the conclusion that creatine is formed normally in the liver by the methylation of glycocyamine. It would be surprising if this is a fortuitous coincidence of little or no physiological significance. Other mechanisms and other sites of creatine formation are, of course, not excluded by these findings.

The significance of methionine here is less clear. Our findings suggest that it is the methylating agent of glycocyamine in liver or its precursor. We are hesitant at present to accept methionine itself as the methylating agent because its effect is small considering the concentration used. It may be that the actual methylating agent is the product of the oxidative deamination of methionine. This and other possibilities are now under investigation.

Du Vigneaud and coworkers (9) have shown that the presence of choline in the diet enables the animal to utilize homocystine or homocysteine in place of methionine. They have suggested that choline enables the body to methylate the sulfhydryl group of homocysteine by furnishing the required methyl group, and further that methionine may furnish the methyl groups of choline. The reality of the latter relationship is now established by direct proof recently presented by du Vigneaud.[1]

Our observations suggest that the S-methyl group of methionine may participate also in the formation of the N-methyl group of creatine. In this connection and in view of our findings with dog liver slices it is interesting that Stekol and Schmidt (10) found

[1] Verbal communication at the meeting of the American Society of Biological Chemists at New Orleans, March 13–16, 1940.

in the dog an increase in urinary creatinine after *dl*-methionine was fed, and none after glycine, glutamic acid, or *l*-pyrrolidonecarboxylic acid.

SUMMARY

1. A survey was made of the possibility of creatine formation *in vitro* by slices of the livers and kidneys of the cat, dog, guinea pig, frog, pigeon, rabbit, and rat.

2. Evidence of the existence of small amounts of creatine precursors in the livers of the guinea pig, pigeon, and rat, and in the kidneys of all of the above animals except the pigeon was found.

3. This finding appears to be much less significant quantitatively than the much more active glycocyamine-methylating function of the liver slices of all animals investigated. Only guinea pig and pigeon kidney slices possessed this ability.

4. The above findings are interpreted as indicating that creatine normally is formed by the methylation of glycocyamine in the liver.

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