THE FORMATION OF CREATINE FROM GLYCOCYAMINE IN THE LIVER

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The study of the precursors of creatine in animals has been beset by two difficulties principally. One has been the lack of really adequate biological material; the other, the lack of a specific, and at the same time sensitive analytical method. Experiments hitherto have consisted in attempts to change the urinary excretion of creatine and creatinine, or the creatine content of the tissues of intact animals or of isolated perfused organs. The normal, *i.e.* uncontrolled, fluctuations in tissue composition and urinary excretion are relatively large compared with the changes induced experimentally; it is often impossible to distinguish when experimental effects are observed, whether these have arisen from changes in the processes of excretion or synthesis; there may be variations in the water content of the tissues, thereby affecting their percentile composition; all of these have stood in the way of firm conclusions being drawn.

Some of these difficulties have been avoided in perfusion experiments on isolated organs (1). These experiments are extremely laborious, time-consuming, and costly. The experimentally induced change in creatine content is at the most—about 30 per cent—a small deviation from the normal. The normal base-line is not constant but varies with the age and weight of the animal. Since the same specimen cannot be used for the experiment and control, a large number of animals must be used first to establish the normal base-line, encompassing the variations of the normal, and then an equally large number for each single experiment in order to obtain a body of data sufficiently large for a statistical conclusion which may be significant.

In 1935 we published some observations of a slight increase in in "apparent" creatine when rat liver slices were incubated with a protein hydrolysate (2). Granted that such liver slices could synthesize creatine, most of the difficulties in working with whole animals or whole organs are avoided. With tissue slices the one specimen of tissue provides material for controls and for testing a variety of experimental variants simultaneously. This was the reason that the observation of even a slight synthesis of creatine by tissue slices seemed promising.

We were faced here, however, with the second difficulty mentioned above, uncertainty whether the material which is augmented by the liver slices is really creatine. A number of substances give a positive test with the Jaffe reagent. The amount of the material in question formed by the liver slices was too small to be identified by isolation.

Accordingly further study of this problem was postponed until a more specific analytical method which could be adapted to submicro scale was available. Such a method was provided by Dubos and Miller (3). They discovered and succeeded in culturing a soil bacterium which specifically destroys creatine and creatinine.

We again took up the problem, employing tissue slices and this new adjuvant to our former submicromethod for creatine (4). We have found that liver slices of the cat, rabbit, and rat are able to convert glycocyamine to creatine. In the experimental conditions we have observed, the increase is 5 to 20 times the amount originally present in the slices. The difficulties and uncertainties which exist in conclusions resting on statistical analysis of small differences have therefore been overcome. The liver is well suited to this study, because its normal creatine content and therefore the control or blank value is very low.

We have found further that when methionine is present in the Ringer's solution, the amount of creatine formed from the added glycocyamine was on the average about 50 per cent greater than when methionine was not added. We have tested thirty-two other substances including amino acids, methylated amines and a purine, and betaine. All of these were negative in this respect. It seems reasonable to conclude therefore that rat liver slices transfer the methyl group of methionine to glycocyamine, thus converting the latter substance to creatine.

Technique

The tissue slice technique used is only slightly modified from that described by Warburg (5). The details of the reaction vessels and the manner in which they are mounted are described in a previous communication (6).

The blank controls and each experimental variant were carried through in triplicate. We have had twenty to thirty reaction vessels, each containing three slices from the same liver, running simultaneously. It was necessary to make the sampling of the slices as uniform as possible, because the capacity to synthesize creatine may vary significantly between the lobes. The procedure we finally adopted was to arrange the reaction vessels in three rows (when each experimental variant is carried through in triplicate), one vessel in each row for each variant. Consecutive slices were transferred to a Petri dish containing Ringer's solution, one slice for each vessel in the row. After 3 minutes soaking in this vessel, with gentle agitation, the slices were transferred to the reaction vessels. The same was done for the other two rows. The whole procedure was then repeated according to the number of slices wanted in each vessel. The reason for the 3 minutes soaking in the Ringer's solution before transfer to the reaction vessels is that more creatine was formed than when the slices were transferred directly with only momentary rinsing in Ringer's solution.

At the end of the reaction period the contents of the vessels, including the slices, were transferred to test-tubes, and the bottles and transfer pipette washed with two 1 ml. portions of 0.02 N HCl. The test-tubes were placed in a boiling water bath for 10 minutes with occasional stirring or shaking. After this time the test-tubes were cooled and the volumes made up to 6 ml. with 0.02 N HCl. These test-tubes are marked at 6 ml. Usually only 0.1 to 0.2 ml. was needed to bring the volumes to the mark. The suspensions were then filtered. The coagulated slices with the coagulated protein in each vessel were transferred to small glass dishes and the weight determined after drying at 100°.

The analytical procedure was essentially that described previously (4). Some minor details were varied from time to time in addition to employment of the bacterial digestion procedure of Dubos and Miller. Table I is a protocol of an experiment in

which the complete analytical procedure was used. Instead of triplicate reaction vessels, six were used for each experimental variant. The boiled extracts in pairs of vessels were joined and filtered. To 8 ml. of this filtrate were added 2 ml. of 0.25 M phosphate buffer at pH 7.0. This solution was divided into two portions. In one-half the creatine was determined directly. The creatine in the other half was destroyed by a suspension of NC soil organisms prepared according to the prescription of Dubos and Miller. The difference between the color developed by the Jaffe reagent with and without this digestion gave the true creatine (and creatinine) in the original solution.

The bacterial digestion was carried out as follows: 5 ml. of the solution neutralized with phosphate were transferred to a 250 ml. Erlenmeyer flask. To this was added 1 ml. of a suspension of the NC soil organisms. The necks of the flasks were covered with squares of Parafilm and set away in an air bath at 38° for $\frac{1}{2}$ to $\frac{3}{4}$ of an hour. At the end of this time 1 ml. of 0.5 N HCl was added to each flask. The contents were then centrifuged. 6 ml. of the clear supernatant solution were taken for analysis. The length of time the bacteria were allowed to react on the experimental solutions was based on a prior determination of the potency of the bacterial suspension used. It was the time required by 1 ml. of the bacterial suspension to destroy completely the creatine in 5 ml. of a 2 mg. per cent solution. This was a larger amount of creatine than in any of the tissue extracts submitted to digestion.

To the 5 ml. of tissue extract containing the phosphate buffer, but which had not been digested by the bacteria, were added 1 ml. of water and 1 ml. of 0.5 N HCl. 6 ml. were then taken for analysis. From this point on the analytical procedure was identical for the solutions which had been digested with bacteria and those which had not. Both sets were carried through to the completion of the analysis simultaneously.

The 6 ml. aliquots were transferred to thick walled Pyrex test-tubes with internal dimensions of 125 × 12 mm. The tubes were covered with parchment paper caps and autoclaved for 20 minutes at 125°. After they were cooled, a small amount of Lloyd's reagent was added to each. We have found that the amount of the Lloyd's reagent may vary from 10 to 60 mg. without affecting the final result. The test-tubes were now shaken for 7 minutes

on a shaker of the type devised by Fisher and Wilhelmi (7). The tubes were then centrifuged, the supernatant solution discarded with the last drop at the rim of the test-tube taken up with filter paper, and the Lloyd's reagent resuspended in 2 ml. of 0.01 N HCl. The tubes were centrifuged again, the supernatant solution again discarded, and the adherent moisture on the walls of the test-tube carefully taken up with filter paper. 3 ml. of a sodium picrate solution were added to each tube. This solution consists of 10 parts of saturated picric acid (purified) and 1 part of 10 per cent NaOH, these being mixed immediately before use. The test-tubes were again shaken for 7 minutes and centrifuged. The color was measured on a spectrophotometer with light of approximately 0.525 μ wave-length. With concentrations ranging from 0 to 2 mg. per cent there is a strictly linear relation between concentration of creatine and the intensity of color measured in this manner.

The following controls were taken through the above identical procedure including digestion by the bacteria: Ringer's solution alone, Ringer's solution containing the same concentration of glycocyamine used in the experiment, and Ringer's solution containing glycocyamine and methionine. Each of these was carried out in triplicate. In addition 1 ml. of the bacterial suspension alone and bacterial suspension plus 5 ml. of 2 mg. per cent creatine were incubated with the experimental solutions and carried through the same analytical procedure, performed in duplicate. Finally a set of five standard creatine solutions with concentrations ranging from 0.1 to 2 mg. per cent was treated in exactly the same manner as the experimental solutions except that they were not submitted to bacterial digestion. The amounts of creatine in the experimental solutions and controls were determined by interpolation from the straight line given by the readings of these standard solutions. The above controls and the standards were carried through afresh in every experiment with the experimental solutions.

It is a testimony to the convenience of this analytical method that we have frequently carried through more than 60 individual analyses from the bacterial digestion to the final spectrophotometer reading in less than 5 hours.

We found in experiments with rat and rabbit liver that essen-

TABLE I

Formation of Creatine by Rat Liver Slices in 6 Hours at 37.5° from Glycocyamine

Treatment	Composition of medium			Dry weight of tissue	Apparent creatine				Residual chromogenic material after bacterial digestion	True creatine			
	Ringer's solution	Glycocyamine	*dl*-Methionine		Total	Total amount formed	Amount formed from glycocyamine	*Q* (creatine) × 100 from glycocyamine		Amount present	Total amount formed	Amount formed from glycocyamine	*Q* (creatine) × 100 from glycocyamine
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
	ml.	*mg.*	*mg.*	*mg.*	*mg. per cent*	*mg. per cent*	*mg. per cent*		*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	
Analyzed immediately	4			40.8	0.12				0.07	0.05			
" "	4			41.5	0.14				0.07	0.07			
" "	4			59.8	0.17				0.10	0.07			
Incubated 6 hrs.	4			34.1	0.21	0.12			0.14	0.07	0.03		(0.15)
" 6 "	4			40.2	0.26	0.14			0.18	0.08	0.03		(0.13)
" 6 "	4			38.0	0.22	0.12			0.14	0.08	0.03		(0.15)
" 6 "	4	0.25		35.4	1.20	0.60	0.38	1.9	0.70	0.50	0.45	0.42	2.1
" 6 "	4	0.25		45.0	1.54	0.94	0.66	2.5	0.72	0.82	0.77	0.74	2.8
" 6 "	4	0.25		35.5	1.31	0.71	0.49	2.4	0.72	0.59	0.54	0.51	2.5
" 6 "	4	0.25	1.45	32.5	1.54	0.90	0.70	3.7	0.72	0.82	0.78	0.75	3.9
" 6 "	4	0.25	1.45	37.8	1.54	0.90	0.64	2.9	0.70	0.84	0.79	0.76	3.4
" 6 "	4	0.25	1.45	35.5	1.53	0.89	0.67	3.2	0.71	0.82	0.77	0.74	3.6
" 6 "	4	0.25			0.61				0.61				
" 6 "	4	0.25			0.60				0.60				

Incubated 6 hrs.	4	0.25			0.60				0.61			
" 6 "	4	0.25	1.45		0.65				0.64			
" 6 "	4	0.25	1.45		0.66				0.63			
" 6 "	4	0.25	1.45		0.61				0.64			
1 ml. bacterial suspension incubated 45 min. at 37°									0.01			
0.1 mg. creatine incubated with 1 ml. bacterial suspension 45 min. at 37°									0.007			
									0.006			
									0.006			

The factor converting mg. per cent to Q(creatine) is $4/100 \times 1.5 \times 171 \times 1/6 \times 1/$(dry weight of tissue): 4/100, since there were only 4 ml. in each reaction vessel for the amount of tissue indicated; 1.5 because these 4 ml. were diluted to 6 ml. in the transfer from the reaction vessels to the test-tubes in which they were boiled; 171, because 1 mg. of creatine would occupy 171 c.mm. at S.T.P. if it were a gas; 1/6, because the experiment was run for 6 hours. Q(creatine) is therefore the amount of creatine formed, expressed as if it were a gas in c.mm. at S.T.P., per mg. of tissue (dry weight) per hour.

tially the same result was obtained whether bacteria were used or not. Accordingly we later dispensed with the use of the bacteria, except as indicated.

Results

Table I is a condensed protocol of a typical experiment. The figures in Column 6 are the spectrophotometer readings converted to mg. per cent by interpolation from the standard curve. The figures in Column 7 are obtained by subtracting from those in Column 6 the values of the glycocyamine or glycocyamine plus the methionine blank, and the amount present in the tissue at zero time. The glycocyamine blank value is quite large. It arises from the conversion of glycocyamine to glycocyamidine during the autoclaving. It was essential that the glycocyamine blanks be treated exactly the same as the experimental solutions through all the operations from immersion in the water bath at 37.5° for the same length of time to the final development of the color. The figures in Column 8 are obtained by subtracting from those in Column 7 the amount of chromogenic material formed by the tissue in the Ringer's solution without glycocyamine. Q(creatine) (Columns 9 and 14) is the amount of creatine formed, expressed as if it were a gas in c.mm. at S.T.P., per mg. of tissue (dry weight) per hour. The figures in Column 11 are the differences between those in Columns 6 and 10. From the figures for the blanks in Columns 6 and 10, it is seen that the bacteria digested none of the glycocyamine. The figures in Columns 9 and 14 are not significantly different. They are a little higher in Column 14 than in Column 9 because in Column 7 a correction should have been applied to the glycocyamine blank value for the glycocyamine converted to creatine, from 10 to 16 per cent. If this had been done, the figures in Column 8 would have been increased by 0.06, which would have made them the same, within experimental error, as those in Column 13.

In a previous determination we have found that there was practically no creatinine in the tissue extracts.

Table I shows that the liver slices convert glycocyamine to creatine. This, as far as we know, is the first time the biological conversion of glycocyamine to creatine has been demonstrated by an unequivocally specific analytical method for creatine, and in which

the tissue used both in the controls and in the experiment came from the identical organ specimen. The increases in the experimental vessels were from 10 to 15 times the amount present in the tissue at the beginning of the experiment. The figures in Columns 9 and 14 show that with added methionine there was 40 per cent more creatine formed than when methionine was not added.

Most of the chromogenic material in the liver slices at the beginning and end of the experiment was not true creatine. This is in accord with the findings of Baker and Miller (8). There is, however, a slight increase in true creatine in the liver slices suspended in Ringer's solution containing no glycocyamine. We have found this repeatedly. Most of this non-creatine chromogenic material found in the tissue blanks is also not glycocyamine. This was ascertained by a direct determination for glycocyamine.

The values for Q(creatine) are much smaller than those found with liver slices for the formation of urea, amino acids, or hippuric acid (6). Nevertheless, this rate, small though it is, is more than sufficient to account for the total creatine plus creatinine excretion in the rat. Thus, an adult rat with a liver weighing 12 gm. might excrete 9 mg. of creatine plus creatinine in 24 hours (9). A Q(creatine) of 0.02 would correspond in such an animal to the formation of 7 mg. of creatine in 24 hours.

The following compounds and combinations of compounds were tried instead of glycocyamine to determine whether they could serve as precursors of creatine: arginine, arginine plus glycine, arginine plus glycolic acid, choline, glycine, glycine plus urea, glycolic acid, and methionine. Each of these has been tested on both rat and rabbit liver slices several times. The results have been consistently negative.

Table II is a summary of most of our experiments with glycocyamine and methionine. In the course of these experiments we have used glycocyamine from two different commercial sources, and two specimens of *dl*-methionine, one prepared in this laboratory and one obtained commercially. A different animal was used for each pair of figures.

The data in Table II show the increase in creatine formation invariably obtained when methionine was added to the glycocyamine. Approximately the same relative increase occurred regardless of age, sex, and nutritional condition of the animal, and

TABLE II

Formation of Creatine by Rat Liver from Glycocyamine with and without Added Methionine at 37.5°

From glycocyamine alone	From glycocyamine and methionine	Duration of incubation	Age and sex	Nutritional condition
Q (creatine) × *100*	*Q (creatine)* × *100*	*hrs.*		
3.2	6.2	1	Adult. ♂	Normal nutrition
6.1	9.0	2	" ♂	" "
4.0	5.5	3	" ♂	" "
4.0	6.5	3	" ♂	" "
5.7	8.1	3	" ♂	" "
1.9	3.4	3	3 mos. ♂	" "
4.2	7.4	3	3 " ♂	" "
6.6	7.9	3	3 " ♀	" "
6.5	9.0	3	3 " ♀	" "
4.6	6.6	4	Adult. ♂	" "
8.5	11.3	4	" ♂	" "
6.1	8.5	4	" ♂	" "
7.3	10.6	4	" ♂	" "
7.7	11.1	4	" ♂	" "
3.1	5.8	4	1 mo. ♂	" "
2.3	4.5	4	1 " ♂	" "
1.5	2.3	4	Adult. ♂	Fasted 66 hrs.
1.5	2.4	4	" ♂	" 66 "
4.1	6.4	4	" ♀	Normal nutrition
4.4	6.9	4	" ♀	" "
2.7*		6	" ♂	" "
2.8*	4.8*	6	" ♂	" "
2.5*	3.6*	6	" ♂	" "
2.4	3.3	6	" ♂	" "
3.1		6	" ♂	" "
2.5		6	2 mos. ♂	" "
2.2		6	Adult. ♀	" "
2.7		6	" ♀	" "

Each of the above figures is the average of a triplicate determination. Each reaction vessel contained 20 to 40 mg. (dry weight) of liver, 4 ml. of Ringer's solution containing 0.25 mg. of glycocyamine, and in the methionine series in addition 1.49 mg. of the amino acid.

* Values obtained with the employment of bacteria in the analysis.

regardless also of the duration of the experiment. The over-all average value for *Q*(creatine) × 100 from glycocyamine alone is 4.1, and from glycocyamine plus methionine 6.6.

We have no explanation as yet for the large variations in the rate of creatine formation in different experiments.

It will be noted that most of the results in Table II were obtained without the employment of bacteria in the analysis. Three experiments with rat liver and three with rabbit liver were carried out in which bacteria were used. The values for Q(creatine) were, as in Table I, essentially the same with and without the use of bacteria. We feel therefore that the results obtained without the use of bacteria with this tissue and under these experimental

TABLE III

Rate for Formation of Creatine from Glycocyamine with and without Added Methionine

Time	Without methionine		With methionine	
	Amount of creatine formed per 100 mg. (dry weight) of tissue	Q(creatine) × 100	Amount of creatine formed per 100 mg. (dry weight) of tissue	Q(creatine) × 100
hrs.	*mg.*		*mg.*	
1	0.018	2.8	0.036	6.2
2	0.070	6.0	0.101	8.6
4	0.095	4.6	0.150	6.4
6	0.144	4.5	0.203	5.8

Each of these figures is the average of a triplicate determination. The composition of the Ringer's solution and amount of tissue were the same as in the experiments of Tables I and II. Two lobes of liver were used. Consecutive slices were placed alternately in the vessels with and without methionine. The comparison at each hour, therefore, is of the activity of immediately adjacent sections of liver. Bacteria were not employed in the analyses here.

conditions are as reliable indices of true creatine as those obtained with bacteria.

Table III contains the results in more detail of an experiment in which the rate of creatine formation from glycocyamine, with and without methionine, was studied. The figures show that the methylation of glycocyamine proceeds unchecked for at least 6 hours. The maximum at 2 hours in each series is accidental. It did not occur in other similar experiments.

The absolute amount of glycocyamine methylated was not increased by a 10-fold increase in the initial concentration of gly-

cocyamine. We have not yet explored thoroughly the effect of changing the concentration of methionine. In one experiment 0.0025 M methionine was as effective as 0.01 M. The fact that addition of so much methionine increases the rate of methylation only 40 or 50 per cent leads us to suspect that the methylating agent in the liver itself may not be methionine. Another piece of evidence which points in this direction is that the ratio of the rates of creatine formation with and without methionine is nearly the same throughout the whole period of from 1 to 6 hours (Table III). In other words, the effects of the methionine and of the methylating agent in the tissues were additive. It is possible that the methylating agent in the tissues is derived from methionine.

The following compounds were tested with rat liver as possible methylating agents of glycocyamine. All gave negative results: acetylcholine, *d*-alanine, *d*-arginine, *l*-asparagine, *l*-aspartic acid, betaine, caffeine, choline, *l*-cysteine, *l*-cystine, ethanolamine, *d*-glutamic acid, *d*-glutamine, glycine, glycolic acid, guanidine, *l*-histidine, *dl*-isoleucine, *l*-leucine, *d*-lysine, mono-, di-, tri-, and tetramethylamine, *d*-ornithine, *l*-hydroxproline, *dl*-phenylalanine, *l*-proline, *dl*-serine, *d*-threonine, *l*-tryptophane, and *l*-tyrosine. The final concentration of glycocyamine in the Ringer's solution in these experiments was always approximately 0.0005 M, and 0.0025 M of the compound whose methylating possibilities were being tested. The significance of the positive effect invariably obtained with methionine is heightened obviously by the fact that all of the above compounds were negative.

Some experiments have been made with the kidneys of the cat, rabbit, and rat. Slices of the cortex with and without methionine either failed to methylate glycocyamine or the slight positive results were within the experimental error. These experiments are part of a survey not yet completed of the organs of a number of animals. The details of these experiments will be published later.

Minced liver of the rat or rabbit failed to give any measurable increase in creatine in 6 hours at 37.5° on incubation with glycocyamine, with or without methionine.

Similarly negative results were obtained with slices of heart

and sartorius. But the cell structure is not preserved in slices of these muscular structures as it is in slices of liver.

Until conditions are discovered in which positive results are obtained with minced liver, we feel that no significance can be attached to negative results with sections whose cell structure has been broken or to extracts of other organs.

DISCUSSION

Beard, Boggess, and Pizzolato (10) proposed that glycine and urea condense to form glycocyamine, which is then methylated by more glycine or glutamic acid. We have observed neither this condensation nor the proposed methylating reaction with rat or rabbit liver slices. The conclusions of Beard *et al.* are based largely on experiments on the rat. The negative results in our experiments with glycine and urea we believe are significant in view of the positive results with glycocyamine and methionine. Experiments on the synthesis of urea, amino acids, and hippuric acid have shown that results obtained with tissue slices afford reliable qualitative information, at least, regarding the potentialities of the intact tissue *in situ*.

Bodansky (11) fed glycocyamine to normal rats and at 3, 6, 12, and 24 hours after feeding measured the glycocyamine and creatine concentrations in the liver, muscle, heart, and kidney. Bodansky's interpretation of the data obtained in these experiments was that significant increases in creatine content occurred only in the kidney, and that the increases in the liver were not sufficiently clear cut to be significant. Bodansky concluded that his findings suggest "that methylation of the guanidoacetic acid may have occurred in the kidneys. . . In view of the occurrence of guanidoacetic acid in large amounts in the liver and the failure to show an increase in creatine, it is surmised that the liver plays an insignificant role, if any, in creatine production."

This surmise is in direct conflict with our observations. Bodansky's experimental observations, however, and ours are not in conflict. Thus the creatine content of the liver in two controls in Bodansky's experiments was 16.0 and 20.4 mg. per 100 gm. of tissue, and in three experiments with glycine, 18.6, 16.8, and 19.0 mg. After glycocyamine feeding the figures are 21.7 mg. in 3 hours

and 23.3 in 6 hours, and in a second experiment 30.5 in 3 hours and 22.3 in 6 hours. The relative increases over the control values in the liver with glycocyamine were of the same order of magnitude as those found in kidney. The increases in true creatine in the liver were probably relatively much larger, since most of the chromogenic material in the liver with the Jaffe reagent is not creatine (Table I, and also Baker and Miller).

There is another physiological factor which must be taken into account in interpreting the data obtained by Bodansky. This factor is that the kidney is better able to store creatine than the liver. The analyses of Baker and Miller and our own show that the true creatine content of the kidney is 4 or 5 times that of the liver. Bodansky found that when creatine was fed the creatine content of the liver was twice the control value at the 3rd hour but had declined to the control value by the 6th hour, whereas in the kidney the concentration was 70 per cent above the control value at the end of the 6th hour.

Our observations show conclusively that glycocyamine can be methylated by rat liver. The rate of methylation by kidney slices is much slower than in liver, if it is not absolutely negative.

All these observations are brought into accord by the hypothesis that in the experiments of Bodansky the creatine synthesized from glycocyamine in liver was quickly removed by the blood and stored for a relatively long period in the kidney. We have no reliable data of our own at present on the possible conversion of glycocyamine to creatine in other organs or in the muscles.

Fisher and Wilhelmi found that when isolated male rabbit heart was perfused there was an increase in creatine when arginine was added to the perfusate. No increase in creatine was observed under these conditions in the hearts of prepubertal animals. Davenport, Fisher, and Wilhelmi (12), extending these observations, found that glycolic acid was essential for the methylation of glycocyamine. They suggested the following mechanism of creatine formation in the rabbit heart. Arginine is broken down to glycocyamine and glycolic acid; the glycolic acid then methylates the glycocyamine to form creatine.

In rat and rabbit liver slices the results with arginine, with and without glycolic acid or glycine, and with the two acids alone did

not yield detectable amounts of either glycocyamine or creatine. The only substance we have yet found which is capable of methylating glycocyamine is methionine.

We have attempted to repeat the observations of Fisher *et al.* with slices of rabbit heart. These experiments were unsatisfactory because of the difficulties of obtaining uniform sampling and because the experimental effects were small compared with the amount of creatine initially present. For the reason stated above we do not attach any significance to these experiments.

The difference between the observations on the perfused heart and ours on the liver stand, for the time being at least, either as an unresolved discrepancy or as indicating important differences in the mechanism of creatine formation in the heart and in the liver.

SUMMARY

1. Liver slices of cat, rabbit, and rat convert glycocyamine to creatine.

2. This methylation is accelerated in rat liver by methionine, (other animals are now being studied).

3. Methionine is the only substance we have yet found among a large number of amino acids, methylated amines, and other compounds which is able to effect this methylation in rat liver.

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