XXXV. THE METABOLISM OF CREATINE

III. THE ROLE OF GLYCOCYAMINE IN CREATINE SYNTHESIS

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IN view of our demonstration [Fisher & Wilhelmi, 1937, 2] that arginine can be converted quantitatively into creatine in the isolated rabbit heart, we have been concerned to discover whether other supposed precursors of creatine can be fitted into a scheme in which arginine is regarded as the primary precursor. Glycocyamine and glycine are the only two substances other than arginine which may reasonably be supposed, on the available evidence, to act as precursors. The early evidence in favour of the formation of creatine from glycocyamine is summarized by Hunter [1928] and more recently Bodansky [1936, 1, 2] has demonstrated increases in creatine excretion and creatine concentration in rat muscle following glycocyamine administration. The evidence in favour of the participation of glycine in creatine synthesis is much less definite, and consists largely of observations on the effect of glycine on the creatine excretion of patients suffering from pseudo-hypertrophic muscular dystrophy (see Milhorat & Wolf [1937] for a full review of this topic). However, Bodansky [1936, 2] could detect no effect of glycine on the creatine excretion or muscle creatine concentration of rats, and Abderhalden & Buadze [1930; 1931] failed to detect any effect of massive doses of glycine on the creatine excretion of dogs.

The positive results obtained with both substances are numerous and definite, and are clearly of greater relative importance than the negative ones. But in neither instance have experiments been so designed that interpretations of the results other than in terms of creatine synthesis are satisfactorily excluded. Nevertheless, it seems that a scheme for the conversion of arginine into creatine which involves these two substances possesses greater plausibility than one which neglects the possibility of their participation in creatine synthesis. A scheme which indicates a possible relation of arginine, glycocyamine and glycine in the process of creatine synthesis is presented below (p. 263).

The reactions leading to the production of glycocyamine and glycollic acid are all of types well known to occur in the mammalian organism, and a similar scheme for the production of glycocyamine was first propounded by Czernecki [1905] and adopted later by Knoop [1910] and by Neubauer [1911]. The respect in which this scheme is new is that it accounts for the methylation of glycocyamine by means of an agent derived from arginine simultaneously with the glycocyamine to be methylated.

This paper is concerned with the experimental test of the hypothesis that glycollic acid can act as methylating agent for glycocyamine in the isolated perfused rabbit heart, since this is the crucial feature of the proposed scheme.

Scheme of reactions involved in the conversion of arginine into creatine

(This paper is concerned with the testing of the part of the scheme enclosed within the dotted lines. The remainder of the scheme is still entirely speculative.)

If glycollic acid can act in this way, then, since glycine can certainly be readily deaminated in the whole organism, and since aliphatic keto- and hydroxy-acids appear to be readily interconvertible, the hypothesis that glycine exercises its influence on creatine synthesis by increasing the supply of methylating agent for glycocyamine is rendered extremely plausible.

In the investigation described below it is shown that the addition of glycocyamine alone or of glycollic acid alone (as the sodium salt) to the perfusate has no effect on the total creatinine of the perfused rabbit heart, but that when both substances are added to the perfusate an increase in total creatinine occurs. Further the amount of glycocyamine disappearing from the perfusion system is negligible when it alone is present in the perfusate, but is considerable and corresponds to the estimated amount of extra creatine formed when glycollic acid is also present.

Methods

The methods used are those described in the preceding papers of this series [Fisher & Wilhelmi, 1937, 1, 2] with the following changes and additions:

(1) Glycocyamine and glycollic acid (neutralized with NaOH) are added to the perfusate in a concentration of 7-11 mg./100 ml.

(2) It has been found advisable to deal with samples and extracts containing glycocyamine as rapidly as possible and in the cold, to prevent changes in the apparent glycocyamine content of the solutions. The perfusion sample tubes are kept in ice until the samples are centrifuged. After the weighing and mincing of the heart, under the protein precipitant, the supernatant liquid is filtered

under suction and transferred to a 100 ml. volumetric flask in ice. The tissue is ground with a stout glass rod, extracted with ice-cold metaphosphoric acid and centrifuged. The supernatant liquid is filtered through the same paper into the same vessel and again transferred to the volumetric flask. The grinding and extraction are repeated seven times.

(3) 10% metaphosphoric acid is used as a protein precipitant instead of trichloroacetic acid.

(4) Estimations of total creatinine and glycocyamine are made on aliquots of a heart extract + perfusate mixture which contains the extract of one-quarter of the heart + one-quarter of the perfusate samples removed during the perfusion + one-quarter of the perfusate left in the apparatus at the end of the perfusion.

(5) Glycocyamine is determined by a method identical in principle with that of Weber [1936] which is described by Bodansky [1936, 2]. Details of the method will be published in a forthcoming paper [Davenport *et al.* 1938].

(6) The data of this paper are derived from the hearts of healthy male rabbits weighing more than 2 kg.

Results

Blank perfusions. In order to determine the limits of accuracy of the analytical methods and to discover whether any significant change in the glycocyamine content of the perfusate occurs when it is kept in oxygenated Ringer-Locke solution at 37° alone or in the presence of glycollate, a number of blank perfusions has been carried out. The results of these perfusions, which are collected in Table I, are very satisfactory considering that analyses have to be carried out on 1/400th of the total creatinine or glycocyamine.

Table I

Creatine added (as creatinine)	Glycocyamine added	Glycollic acid added	Observed appearance of creatinine	Observed appearance of glycocyamine
6.71	—	—	0.18	—
12.50	—	—	0.46	—
7.12	11.00	—	0.05	-0.19
7.68	10.87	—	-0.72	-0.17
9.77	10.62	10.78	—	0.07
9.54	10.51	10.67	—	0.13

(All figures in mg.)

Glycollic acid. The results of nine perfusions in which glycollic acid was added to the perfusate are shown as hollow circles in Fig. 1. The dotted line represents the relation between total creatinine and heart dry weight in controls and glucose-perfused hearts [Fisher & Wilhelmi, 1937, 2]. There is no regular or serious deviation of the glycollic acid points from the control line, and we conclude that glycollic acid alone does not cause any significant increase in the total creatinine of the heart + perfusate.

Glycocyamine. The solid circles in Fig. 1 illustrate the effect of glycocyamine on the total creatinine of the heart. Here again, it is clear that there is no significant increase in total creatinine of the heart.

Glycocyamine + glycollic acid. Fig. 2 illustrates the relation between total creatinine and dry weight in hearts perfused with glycocyamine and glycollic acid. The interrupted line is the control regression line and the continuous line is the regression line for the present experimental data. It is clear that the total creatinine of the heart is increased by perfusion with these two substances.

These three groups of perfusions therefore give good reason to believe that glycollic acid and glycocyamine react in the heart to yield creatine.

Fig. 1.

Fig. 2.

However, this evidence is purely qualitative in character, and we have therefore carried out experiments to determine the effect of glycollic acid on the disappearance of glycocyamine from the heart + perfusate.

Glycocyamine disappearance. The results of determinations of glycocyamine disappearance from the perfusion system, in the presence and absence of glycollate, are collected in Table II. The column headed ΔG gives the amount of creatinine in mg. to which the amount of glycocyamine disappearing is equivalent, and the column headed ΔC gives the most probable value of the extra total creatinine.

ΔC is calculated as follows:

The relation between total creatinine (Y) and heart dry weight (x) for 45 post-pubertal control and glucose-perfused hearts [Fisher & Wilhelmi, 1937, 2] is $Y = 4.01x + 3.19$. The relation between these variables derived from the 21 observations on hearts perfused with glycocyamine + glycollate is $Y = 5.05x + 2.93$. Since these two relations give the most probable values for the amounts of total creatinine present in a heart of a particular dry weight before and after perfusion with glycocyamine + glycollate, it follows that

$$\begin{aligned}\Delta C &= (5.05 - 4.01)x + 2.93 + 3.19 \\ &= 1.04x - 0.26.\end{aligned}$$

Since ΔC is merely an approximate estimate, the individual values of $\Delta G/\Delta C$ have little significance. However, provided that the errors in estimation of ΔC are purely random ones, the mean of a series of estimates of $\Delta G/\Delta C$ should approximate closely to unity if a quantitative relation exists between glycocyamine disappearance and creatine appearance. We are therefore of the opinion that the mean value of 1.03 ± 0.16 obtained for the present series of perfusions is a strong indication of quantitative conversion of glycocyamine into creatine. This opinion is strengthened by the fact that the mean glycocyamine disappearance in the absence of glycollate is not significantly different from zero, and is

only one-eighth of the mean disappearance in the presence of glycollate, although the mean sizes of the hearts used in the two series of perfusions are similar.

Table II

	Perfusion	Heart dry wt. (g.)	Glyco- cyamine disappear- ance mg.	ΔG	ΔC	$\Delta G/\Delta C$
Perfused with glycocyanine alone	143	1.03	0.23	—	—	—
	145	1.21	0.17	—	—	—
	152	1.08	0.46	—	—	—
	153	1.34	0.22	—	—	—
	154	1.19	0.03	—	—	—
	Means	1.17	0.13 $\pm$ 0.11			
Perfused with glycocyanine and glycollic acid	142	1.12	1.04	1.01	0.90	1.12
	144	1.34	1.40	1.35	1.13	1.20
	146	2.09	2.17	2.09	1.91	1.09
	155	1.14	0.94	0.91	0.93	0.98
	156	0.96	0.52	0.50	0.74	0.68
	157	0.92	0.93	0.90	0.70	1.29
	158	0.99	1.66	1.60	0.77	2.08
	159	0.85	0.28	0.27	0.62	0.44
	160	0.90	0.77	0.75	0.68	1.10
	162	1.11	0.31	0.30	0.89	0.34
	Means	1.14	1.00 $\pm$ 0.19			1.03 $\pm$ 0.16

Preformed creatinine of the heart. A series of determinations of the preformed creatinine content of hearts is collected in Table III, and it is apparent that there is no significant change in the preformed creatinine of the heart in response to different treatments, and, further, that the order of magnitude of the preformed

Table III. *Preformed creatinine content of rabbit hearts*

Animal	Treatment of heart	Preformed creatinine mg.	Total creatinine mg.	$\frac{\text{Preformed} \times 100}{\text{Total}}$
107	Arginine	0.32	10.73	3.0
108	Arginine	0.22	5.45	4.0
159	Glycocyanine + glycollate	0.35	7.76	4.5
160	Glycocyanine + glycollate	0.21	7.95	2.7
162	Glycocyanine + glycollate	0.32	8.11	4.0
153	Glycocyanine	0.32	9.40	3.3
154	Glycocyanine	0.43	—	—
62 C	Control	0.21	9.80	2.1

creatinine is less than that of the increase in total creatinine brought about by perfusion with glycocyanine and glycollate. It is therefore unnecessary to consider the hypothesis that glycocyanine may be converted into creatinine in the heart.

We regard these results as adequate to demonstrate that glycollic acid can act as an agent for the methylation of glycocyanine in the isolated perfused heart with the production of creatine.

The data on which Figs. 1 and 2 are based are collected in Table IV.

Table IV

Perfusion number	Heart dry weight g.	Glycocyamine added mg.	Glycollic acid added mg.	Total creatinine of heart + perfusate mg.
126	1.26	—	7.9	9.57
135	1.57	—	12.8	9.84
136	0.86	—	10.3	5.54
137	0.88	—	11.4	4.39
138	0.97	—	10.5	7.16
139	1.07	—	11.7	6.71
140	1.70	—	11.4	9.84
141	1.03	—	10.8	6.93
147	1.48	—	11.7	9.32
55	0.84	11.0	—	5.41
115	1.02	10.8	—	5.01
116	1.30	10.5	—	8.09
117	1.41	10.5	—	7.53
118	1.08	10.8	—	6.87
143	1.03	10.2	—	6.94
145	1.21	10.2	—	7.72
152	1.08	10.9	—	7.33
153	1.34	11.1	—	9.40
120	1.06	12.5	8.7	9.55
121	0.99	11.2	15.0	6.88
122	1.24	11.1	10.1	7.89
123	1.23	10.7	11.4	8.00
124	1.73	10.6	10.0	10.84
125	1.39	11.0	9.0	9.70
127	1.33	11.7	12.0	9.34
128	1.44	10.3	9.1	10.35
129	1.60	10.5	7.8	11.51
131	1.47	10.7	10.7	10.51
133	0.76	10.8	9.0	6.85
134	1.54	11.7	10.1	11.37
142	1.12	11.1	11.5	10.40
144	1.34	10.3	10.5	9.03
146	2.09	10.7	9.9	14.13
155	1.14	10.8	12.3	9.01
157	0.92	10.7	11.9	7.97
158	0.99	10.9	12.2	7.56
159	0.85	10.5	11.7	7.55
160	0.90	10.8	11.8	7.73
162	1.11	10.7	11.2	7.90

(The figures for the amounts of glycocyamine and glycollic acid added represent the amounts present in the total of 110 ml. of perfusate used in each perfusion.)

DISCUSSION

The evidence presented in this paper and in the previous paper of this series supports strongly the scheme presented in the introduction to this paper.

In the first place, arginine can be converted into creatine in the isolated rabbit heart [Fisher & Wilhelmi, 1937, 2]. Since arginine and creatine both contain a guanidine group it is economical of hypotheses to suppose that this group is preserved during the conversion of arginine into creatine. If this is so, then a process of methylation of the guanidine nitrogen atom which is bound to the side-chain must occur at some stage of the conversion.

Further argument depends on the assumption that glycocyamine can be obtained by the catabolism of arginine. Such a process has not yet been

demonstrated experimentally, but the following points are strongly in favour of its occurrence:

(1) Arginine can be converted quantitatively into creatine in the isolated heart.

(2) Glycocyamine can also be converted quantitatively into creatine in the heart in certain circumstances.

(3) The chemical transformations necessary for the conversion of arginine into glycocyamine are all chemically and biologically plausible.

On these grounds we are strongly inclined to believe that glycocyamine is a product of arginine catabolism in the isolated heart.

If this is so, the methylation of glycocyamine must constitute the ultimate stage of the conversion of arginine into creatine. We have demonstrated that glycocyamine is not methylated by an agent present in the isolated perfused heart, i.e. that there is no significant change in total creatinine when glycocyamine alone is added to the perfusate. It follows that when creatine synthesis occurs from arginine, the arginine must provide the methylating agent as well as the glycocyamine. The fact that glycollic acid is capable of acting as a glycocyamine-methylating agent is important, since glycollic acid is very closely related to glycine, and provides our present justification for bringing glycine into our scheme.

We wish to make here the suggestion that glycollic acid may be the normal methylating agent for glycocyamine, and that the effect of glycine on creatine synthesis may be due to its ability to act as a source of glycollic acid. We believe this suggestion to be a new one. Tripoli & Beard [1934] suggested that glycine might be the source of the *N*-methyl of creatine but without specifying any detailed reaction scheme.

SUMMARY

Neither glycocyamine nor glycollic acid alone increases the total creatinine of the isolated perfused rabbit heart when it is added to the perfusate in a concentration of 7–11 mg./100 ml. When both substances are added to the perfusate the total creatinine is increased, and the increase is due to increase in the creatine content of the heart + perfusate.

There is no significant disappearance of glycocyamine from the perfusion system when it alone is present in the perfusate. In the presence of glycollic acid an amount of glycocyamine disappears which is approximately equivalent to the amount of creatine appearing.

It is concluded that glycocyamine is converted quantitatively into creatine in the perfused heart in the presence of glycollic acid, and that glycollic acid acts as a glycocyamine-methylating agent.

Reasons are given for believing that when arginine is converted into creatine it is first broken down to yield glycocyamine and glycollic acid, and a scheme of possible reactions involved in the conversion of arginine into creatine is presented which includes a suggestion concerning the role of glycine in creatine synthesis.

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APPENDIX

In this paper and an earlier one [Fisher & Wilhelmi, 1937, 2] we have been compelled by the nature of our material to calculate the quantitative relations between the amount of precursor disappearing and the amount of creatine synthesized by having recourse to a quantity ΔC —"the most probable value of the extra total creatinine".

We now have two sets of perfusions in which creatine synthesis has been observed from different precursors. Both sets are plotted in Fig. 3, the glycocyamine+glycollate points being plotted as solid, and the arginine points as

Fig. 3

hollow circles. The total creatinine-heart dry weight relations of the two series are clearly not significantly different, and the pooled regression calculated for the

two series is $Y = 5.38x + 2.55$. This gives a new value of ΔC , namely $\Delta C = 1.37x - 0.64$. The values of ΔC calculated from this relation for each of the perfused hearts, together with the observed values of ΔA and ΔG , and the corresponding ratios, are collected in Table V.

Table V

ΔA	ΔC	$\Delta A/\Delta C$	ΔG	ΔC	$\Delta G/\Delta C$
1.13	1.24	0.91	1.01	0.89	1.13
1.57	1.31	1.19	1.35	1.20	1.13
0.94	0.95	0.99	2.09	2.22	0.94
1.13	1.22	0.93	0.91	0.92	0.99
1.09	1.24	0.88	0.50	0.68	0.74
1.07	1.13	0.95	0.90	0.62	1.45
1.25	1.17	1.07	1.60	0.72	2.22
1.53	1.28	1.20	0.27	0.52	0.48
1.02	0.98	1.04	0.75	0.59	1.27
1.62	1.43	1.13	0.30	0.88	0.34
		1.03 ± 0.04			1.07 ± 0.17

It will be seen that the alteration has no significant effect on the mean ratios in either series, and it follows that the observed mean values are not the outcome of chance correspondence of the calculated relation between ΔC and x with the relation required to show quantitative conversion of precursor into creatine. That is, Table V provides further support for our view that both arginine and glycocyamine can be converted quantitatively into creatine in the isolated heart.