1-18-32 2:30 P.M. The dog sacrificed 77 hours after last injection period. A blood sample was taken at this time with the results given in last column. The serum of this blood sample was highly jaundiced.

Autopsy. The liver was of a pale brownish yellow color, very soft and friable with all the characteristic signs of phosphorus poisoning. Kidneys seemed normal.

These results necessarily must be taken with caution but they do seem to point to a connection between the liver and parathyroid action. The effect of other liver poisons is now being studied.

6053

Studies on Arginine II. Phosphoarginine as a Possible Precursor of Creatine.

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Arginine was first suggested as the mother substance of creatine by Czernecki.[1] From theoretical considerations the hypothesis has been generally regarded as an attractive one. It is in accordance with the fact that arginine, creatine and creatinine are the most abundant of the guanidine derivatives present in the animal organism. It is possible to postulate a series of plausible reactions by which the conversion of arginine to creatine might conceivably be accomplished.[1] It is also in harmony with the mutually exclusive occurrence of arginine and creatine as demonstrated by Kutscher and Ackermann.[2] These investigations have shown that creatine, a characteristic constituent of vertebrate muscle, is replaced by arginine in invertebrate muscle. Indeed the corresponding phospho esters, in which the muscle arginine and creatine largely occur, are even functionally equivalent.[3, 4]

Nevertheless, efforts to demonstrate the origin of creatine from arginine have been unsuccessful, almost without exception. Numerous investigations, described in Hunter's monograph,[5] and others referred to by Hyde and Rose[6] have been entirely negative in result or open to serious criticism upon some crucial point.

1 Czernecki, W., *Z. physiol. Chem.*, 1905, **44**, 294.

2 Kutscher, F., and Ackermann, D., *Z. Biol.*, 1926, **84**, 181.

3 Meyerhof, O., and Lohmann, K., *Naturwissenschaften*, 1928, **16**, 47.

4 Lundsgaard, E., *Biochem. Z.*, 1930, **230**, 10.

5 Hunter, A., *"Creatine and Creatinine,"* Longmans Green, 1927.

6 Hyde, E. C., and Rose, W. C., *J. Biol. Chem.*, 1929, **84**, 535.

Doubtless one of the greatest difficulties in experiments *in vivo* has been the intervention of arginase, as a result of which administered arginine suffers rapid conversion to ornithine and urea. This competitive mechanism leaves little if any arginine available for the various reactions which are alleged to lead to creatine formation.

With this difficulty in mind, we have studied the possible rôle of phosphoarginine as a precursor of creatine. Unlike arginine, phosphoarginine is not acted upon by arginase.[7] In consequence, the arginase mechanism fails to enter as a disturbing factor and there would be, seemingly, every opportunity for phosphoarginine to undergo conversion to phosphocreatine if arginine and creatine are actually related in accordance with the hypothesis mentioned.

Phosphoarginine was prepared by the method of Meyerhof and Lohmann.[7] The large, edible crab *(Cancer magister)* was used as the source. The animals were chilled thoroughly before killing. The legs were rapidly removed, minced by the use of a chilled grinder, and the mincings immediately frozen with liquid air. The frozen material was then triturated with cold trichloroacetic acid and the extract submitted to the barium precipitations recommended by Meyerhof and Lohmann. After one or 2 unsuccessful preliminary attempts at isolation, we found it desirable to omit the final treatment with sulphuric acid and subsequent reprecipitation of the barium salt, since the desired material was being lost, presumably by hydrolysis in the acid solution. In the first successful preparation 175 mg. of the soluble barium salt were obtained from 3 crabs. Two mg. were used for the determination of barium and phosphorus and 3 mg. for arginine. The preparation was found to contain 20% Ba, 9.8% P, and 57% of arginine (theoretical values for pure barium salt of phosphoarginine are Ba, 21.3; P, 9.64; arginine, 54.1). All of the arginine and phosphorus present in the material were in the combined form. The analysis was conducted after preliminary acid hydrolysis in $2N$ H_2SO_4. Barium was determined gravimetrically as $BaSO_4$, and phosphorus (in the filtrate) by the method of Fiske and Subbarow.[8] Arginine was determined in a separate portion of hydrolysate by the method of Kiech, Luck, and Smith.[9]

A second preparation of the salt from 4 crabs yielded 480 mg. of less pure material (35% barium salt of phosphoarginine).

In order to conserve the material available and to extend its use

[7] Meyerhof, O., and Lohmann, K., *Biochem. Z.*, 1928, **196**, 49.

[8] Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1925, **66**, 375.

[9] Kiech, V. C., Luck, J. M., and Smith, A. E., *J. Biol. Chem.*, 1931, **90**, 677.

over as many animals as possible, mice were used as experimental subjects. For at least 3 weeks prior to experiment they received a standard diet of ground cereal grains, whole milk powder, alfalfa meal, bone meal, yeast, sodium chloride, and cod liver oil. For the 24 hours preceding analysis the animals received water only. On withdrawal of the food each animal was placed upon a coarse wire screen over a lightly-paraffined iron mortar, covered with an inverted funnel. Eighty mg. of the barium salt in 1.0 cc of water were treated with an equivalent amount of sodium sulphate (to remove the barium) and diluted to 1.5 cc. The suspension was injected subcutaneously in 3 portions of 0.5 cc. each at 4 hour intervals, the first injection being at the time of food withdrawal. Controls were treated in identical fashion, except that 1.0 cc. of barium chloride was used in place of arginine barium phosphate. Under these conditions the mechanical loss of phosphoarginine was avoided. Barium was also removed without acidification and hence without danger of phosphoarginine hydrolysis.

During the experimental period the animals were kept in a warm room at approximately 28°. Twenty-four hours after the first injection the animals were stunned, then frozen and ground with liquid air in the mortar containing the excreta of the 24-hour period. After being thoroughly powdered, aliquot portions were weighed out and used in the determination of urea (method of Allen and Luck[10]) and total creatinine. For the latter, 4 different methods were tried but only that of Rose, Helmer, and Chanutin[11] was found

TABLE I.

Control Mice				Experimental Mice			
Mouse No.	Wt.	Total Creatinine	Urea	Mouse No.	Wt.	Total Creatinine	Urea
	gm.	mg. per 100 g.	mg. per 100 g.		gm.	mg. per 100 g.	mg. per 100 g.
10	28	171.2	153.6	23	23	138.7	——
11	25	152.6	133.0	24	23	141.0	152.5
12	24	166.3	151.9	25	23	135.6	161.8
13	26	178.9	132.2	26	21	172.3	108.2
14	26	139.8	133.3	27	28	149.0	126.9
15	22	131.9	164.0	28	25	140.6	——
16	22	140.0	135.9	29	26	145.7	143.6
17	21	133.6	150.6				
18	25	144.1	124.6				
19	24	139.6	——				
20	24	143.5	——				
21	23	142.1	152.5				
22	22	145.6	155.3				

Mice Nos. 23, 24 received preparation I of phosphoarginine.
Mice Nos. 25 to 29 received preparation II of phosphoarginine.

[10] Allen, F. W., and Luck, J. M., *J. Biol. Chem.*, 1929, **82**, 693.

[11] Rose, W. C., Helmer, O. M., and Chanutin, A., *J. Biol. Chem.*, 1927, **75**, 543.

to give consistent results and close agreement between duplicate determinations. The amount of phosphoarginine (preparation I) injected was sufficient, if totally converted, to yield 1.2 mg. of creatine (as creatinine) per gram of mouse. This would represent an increase of 90% over the basal creatine-creatinine level. Any appreciable hydrolysis of the phosphoarginine would also be reflected by significant increases in urea, because of the rapid metabolism of arginine. With preparation II of phosphoarginine, 30% increases in total creatinine would be the maximum attainable.

The results presented in Table I demonstrate that under the conditions of these experiments phosphoarginine failed to undergo conversion into phosphocreatine, creatine or creatinine. They do not support the hypothesis that arginine or phosphoarginine is the mother-substance of creatine.

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Influence of Digitalis on the Sensitivity of the Cardiac Vagus Endings.

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The work of Heymans[1, 2] on the mechanism of the bradycardia of digitalis seems to dispose of the older theory that this drug stimulates the vagal centers directly. It supports Straub's[3] suggestion that the slowing is due to a direct myocardial action, the sinus pacemaker becoming more responsive to vagal tone. In support of this viewpoint, Rothberger and Winterberg[4] have claimed that digitalis lowers the threshold to electrical stimulation of the vagus trunks, and Weger has observed[5] that it makes the parasympathetic endings in the intestine more irritable to stimulation by pilocarpine. However, Weiss[6] regards the slowing after digitalis as due to reflexes originating in the viscera innervated by the vagus. In order to obtain data regarding the validity of the first of these theories,

[1] Heymans, J. F., and Heymans, C., *J. Pharm. Exp. Therap.*, 1926, **29**, 203.
[2] Heymans, C., *Ergeb. der Physiol.*, 1929, **28**, 300.
[3] Straub, W., *Heffter's Handb. der Exp. Pharmakol.*, **2**, 1422.
[4] Rothberger and Winterberg, *Arch. f. Physiol.*, 1910, **132**, 233.
[5] Weger, P., *C. R. Soc. Biol.*, 1927, **96**, 803.
[6] Weiss, S., *Med. Clinics of N. Amer.*, 1932, **15**, 963.