marked excretion of PGA in feces, but not in urine, the amount excreted exceeding by far the tissue rise in level of vitamin. It is suggested that biotin administration may influence synthesis of PGA by intestinal microflora and, as a consequence, improve tissue levels of the vitamin.

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Formation of Creatine from Guanidinoacetate in Pancreas.* (25103)

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Several investigators established that 2 enzymes are involved in biosynthesis of creatine (1). Arginine + glycine ⇄ guanidinoacetate + ornithine. Guanidinoacetate + S-adenosylmethionine → creatine + S-adenosylhomocysteine. The first enzyme, arginine-glycine transamidinase, has long been known to occur in mammalian kidney(2), while the second enzyme, guanidinoacetate methylferase, has been found in liver(3,4). Previously it has been assumed that under physiological conditions these 2 synthetic reactions are carried out in successive steps in different organs of the mammal. Guanidinoacetate was believed to be synthesized in the kidney from arginine plus glycine and then transported *via* the blood stream to the liver, where methylation to creatine took place. Recently, however, another mammalian organ has been implicated in creatine biosynthesis, with the discovery that pancreas contains a high concentration of arginine-glycine transamidinase (5). Since guanidinoacetate can be synthesized in pancreas, it became of interest to determine whether methylation of guanidinoacetate to form creatine might also occur in this organ. The purpose of this paper is to present evidence for occurrence of guanidinoacetate methylferase activity in mammalian pancreas.

Methods. The enzyme preparations were dialyzed beef or dog pancreas fractions which precipitate between 30% and 60% saturation with ammonium sulfate, prepared as described elsewhere(5). All incubation mixtures contained pancreas enzyme preparation, 24 mg; tris (hydroxymethyl) aminomethane buffer, pH 7.6, 100 μmoles; other additives as noted in individual experiments; final volume, 1 ml. Tubes were incubated 2 hr at 37°C; the reactions were terminated by adding 1 ml water and heating 3 min at 100°C. In the time-course experiment with guanidinoacetate-2-C^{14}, 5 μliters were removed from each reaction mixture at times indicated and spotted on paper chromatograms. Non-labelled guanidinoacetate and creatine were added to each spot as carriers. After development of the paper chromatograms with buffered phenol, the separated compounds were located with alkaline ferricyanide-nitroprusside spray. Areas of the paper containing creatine and guanidinoacetate were cut out, eluted, dried, and counted. In experiment with glycine-2-C^{14}, non-labelled guanidinoacetate and creatine were added as carriers to the reaction mixtures immediately prior to termination of incubation. Guani-

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TABLE I. Synthesis of Creatine from Various Precursors in Beef Pancreas.

Precursors			μmoles creatine formed
(a) Arginine	.47	μmoles	.21
+ Glycine	1.3	"	
+ S-adenosylmethionine	1.7	"	
(b) Guanidinoacetate	.51	"	.23
+ S-adenosylmethionine	1.7	"	

dinoacetate and creatine were separated from the deproteinized solutions by paper chromatography, and were eluted and counted as described above.

Results. Preliminary experiments were carried out to determine if guanidinoacetate methylferase is present in mammalian pancrease in high enough concentration to be physiologically significant. One way to determine this is to compare activity of pancreas with that of a tissue known to have high guanidinoacetate methylferase activity, *e.g.*, guinea pig liver(3). The tissues to be compared were homogenized, centrifuged, dialyzed, and assayed; the creatine formed was determined by a diacetyl method(6). The relative enzyme activities were as follows: guinea pig liver, 100; dog pancreas, 73; dog liver, 26. These results encouraged the more extensive experiments described below.

The experiment summarized in Table I demonstrates that creatine can be synthesized by beef pancreas preparation from either guanidinoacetate or arginine plus glycine, with S-adenosylmethionine‡ as the methyl group donor. The versatility of pancreas with respect to creatine precursors is clearly apparent in this experiment. In contrast to pancreas, mammalian liver cannot synthesize creatine from arginine, glycine, and S-adenosylmethionine, since liver lacks the enzyme arginine-glycine transamidinase. Although the diacetyl method was employed here for assay of creatine(6), the assay involving conversion to creatinine, adsorption and elution from Lloyd's reagent and color development with alkaline picrate(7), gave similar results in independent experiments.

‡ S-Adenosylmethionine bromide (80% pure) was purchased from California Corp. for Biochemical Research.

Unfortunately, both of the colorimetric assays for creatine mentioned above are relatively non-specific(4). Consequently to establish the presence of guanidinoacetate methylferase in pancreas beyond a reasonable doubt, additional experiments were performed with radioactive substrates. In these experiments reactants and products were isolated by paper chromatography, eluted, and counted (8). Fig. 1 shows time-course of conversion of guanidinoacetate-2-C^{14} to creatine, as catalyzed by beef pancreas preparation, in presence and absence of the methyl group donor, S-adenosylmethionine. No creatine was formed in the absence of added methyl donor.

Table II summarizes an experiment in which glycine-2-C^{14} was employed as a creatine precursor, with arginine as the formamidine group donor and S-adenosylmethionine as the methyl donor. It can be seen that glycine was not incorporated into creatine in significant amounts in the absence of added methyl donor; however, guanidinoacetate was formed from glycine and arginine. On the other hand, when S-adenosylmethionine was present, glycine was converted all the way to creatine, with a lesser amount accumulating in the intermediate guanidinoacetate. This conversion of glycine to guanidinoacetate and to creatine was completely inhibited by ornithine, a powerful inhibitor of arginine-glycine transamidinase(5). Since separate experi-

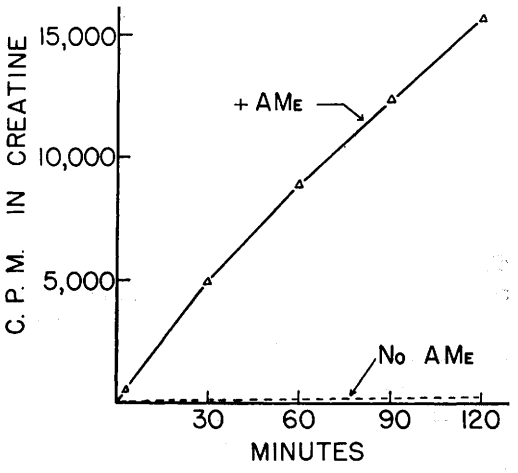

FIG. 1. Time-course of conversion of guanidinoacetate-2-C^{14} to creatine in beef pancreas. Guanidinoacetate, 0.25 μmole, 22,000 cpm; S-adenosylmethionine (AMe), 3.3 μmoles.

TABLE II. Incorporation of Glycine-2-C^{14} into Guanidinoacetate and Creatine in Beef Pancreas.

Compounds added		cpm in Guanidinoacetate × 100	cpm in Creatine × 100
(a) Glycine-2-C^{14}, 37,000 cpm	.27 μmoles	239	2
+ Arginine	.95 "		
(b) *Idem* + S-adenosylmethionine	3.3 "	86	157
(c) " + S-adenosylmethionine	3.3 "	2	5
+ Ornithine	18 "		

ments had established that ornithine does not inhibit guanidinoacetate methylferase activity, these results provide further evidence that guanidinoacetate is an intermediate in the incorporation of glycine into creatine.

Discussion. It is now apparent that mammalian pancreas contains physiologically significant concentrations of both enzymes involved in the biosynthesis of creatine from arginine, glycine, and S-adenosylmethionine. Although it would appear that biosynthesis of creatine might be more efficiently carried out *in toto* in one organ (pancreas) rather than in successive steps in different organs, further work must be done before the relative importance of the 2 pathways can be assessed.

In either event, an important physiological role for pancreas in the biosynthesis of creatine precursor, guanidinoacetate, seems assured(5). Kidney, the other organ capable of participating in guanidinoacetate synthesis, may play only a supporting role in this respect, since its arginine-glycine transamidinase concentration is lower, and its arginase activity is much higher than that of pancreas.

Summary. An enzyme preparation from mammalian pancreas catalyzes the synthesis of creatine from guanidinoacetate and S-adenosylmethionine. Inasmuch as pancreas, in contrast to liver, can also synthesize the creatine precursor, guanidinoacetate, from arginine plus glycine, it would appear that pancreas may play a unique role in biosynthesis of body creatine.

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Modification of Protein Catabolism in the Anuric Dog.* (25104)

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Numerous studies have been reported on urea accumulation and survival time in anuric animals under various conditions. There have been, in general, beneficial effects from carbohydrate feeding and harmful effects from fasting or protein feeding(1). These results are presumably due to modification of the protein catabolic rate in each case. The response to testosterone has been variable, depending on diet used and presence or absence of catabolic accelerators such as tissue necrosis or

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