time or another had shown elevated tail systolic blood pressures. It appears that in addition to the destructive calcific lesion there is a separate histological process of adaptation to high blood pressure going on. Evidence for this is the hypertrophy of elastic tissue elements, with the development of short cross-over bands between concentric elastic lamellae, seen in aortas which did not show the calcific lesions as well as those which did. Development of these changes was associated with alterations of the shape of the stress-strain curve when the aortas were subjected to longitudinal stretch.

Summary. The development and incidence of medial calcific disease of the aorta following operations on the kidneys of male albino rats is described.

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Synthesis of Amino Nitrogen from Ammonia in *Hymenolepis diminuta.** (20857)

JACK W. DAUGHERTY. (Introduced by Roy V. Talmage.)
From the Department of Biology, The Rice Institute, Houston, Tex.

The rat tapeworm, *Hymenolepis diminuta,* is essentially independent of the protein in the diet of its host. Chandler(1) showed that no significant change in worm size, weight, or egg production could be induced under conditions of protein deprivation. Under such conditions it is quite probable that a considerable part of the amino nitrogen needed for growth and egg production comes directly from the host in the form of amino acids diffused from the intestinal mucosa. There is, however, the possibility that other sources of nitrogen contribute to these synthetic mechanisms. An investigation of the ammonia content of the rat intestine showed that a considerable amount was consistently present. The function of ammonia in intermediary protein metabolism is not very well understood; however, several workers have shown that it can contribute to the synthesis of certain amino acids and related substances(2,3). On the basis of these studies on mammalian tissues an investigation of the role of ammonia in the protein metabolism of *H. diminuta* was begun.

Materials and methods. Mature tapeworms, raised in male albino rats of the Sprague-Dawley strain, were infected with *H. diminuta* cysticercoids according to the technic of Chandler(1). The worms were removed from the intestine by flushing with isotonic KCl. They were thoroughly washed and were incubated singly in a Krebs-Ringer-Phosphate (KRP) medium modified by the addition of 0.004 M $(NH_4)_2CO_3$ and 0.1 M sodium pyruvate, 0.1 M sodium alpha-ketoglutarate, 0.1 M sodium oxalacetate, 0.1 M sodium succinate, or 5% glucose. As a means of blocking the tricarboxylic cycle, M/30 sodium malonate was included in several of the experiments in which glucose served as the substrate. The incubations were carried out in air at 38°C. At the end of 90 minutes the worms were removed, weighed, homogenized, and the free amino acids extracted for chromatographic resolution. The paper chromatograms were run on Whatman No. 4 with phenol as the

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TABLE I. Amino Nitrogen in mg Produced by 1 g (Dry Weight) *H. diminuta*/Hour.

	Incubation media	No. of animals	Amino N.
A	KRP	12	.13
B	+ $(NH_4)_2CO_3$	12	1.4
C	+ alpha ketoglutarate	12	4.8
D	+ pyruvate	12	6.3
E	+ oxalacetate	12	7.4
F	+ glucose	8	2.8
G	+ succinate	10	2.4
H	+ glucose + malonate	9	2.6

solvent. Development was with ninhydrin. No attempt was made at this time to estimate quantitatively the individual amino acids. The media were analyzed for free amino nitrogen by the photometric method of Frame, Russell, and Wilhelmi(4). These results were checked by comparison with the ninhydrin method of Van Slyke, MacFayden, and Hamilton(5). *Controls* were run in KRP from which the tissue or substrates were omitted. No amino nitrogen was found when the worms were omitted but a small amount appeared in those tubes in which the worms alone were present. This was assumed to be the result of a diffusion from the worm itself and this amount was subsequently subtracted from the value obtained from the experimental incubation to give a corrected figure. A second type of control involved the use of liver slices as a check on certain of the worm experiments. The results of these incubations were chromatographically analyzed by the procedures described above.

Results. The incubation of *H. diminuta* in the presence of NH_4 ions and various substances associated with glucose metabolism resulted in marked increases in amino nitrogen (Table I). With media containing the salts of alpha-ketoglutaric acid, pyruvic acid, or oxalacetic acid the mean amino nitrogen produced, in mg per g dry weight of worm, was 4.8, 6.3, and 7.4, respectively. It is noteworthy that these results parallel, in general, the results obtained on transaminase activities in the rat tapeworm with regard to the substrate effectiveness of these three keto acids(6). A small amount of amino nitrogen was produced when the NH_4 ions alone were included. This would seem to indicate that ketoacids, comparable with those associated in mammalian tissues with the tricarboxylic cycle, were present in the tapeworm, and that they were capable of acting as acceptors of the ammonia nitrogen. The chromatograms of the worms from this experiment bear out this point (Col. B, Fig. 1); they show that amino acids directly related to some of these keto acids were greatly increased in amount following the incubation with ammonia, *e.g.*, alanine and glutamic acid.

The ability of the tapeworm to utilize glucose as a substrate for amino nitrogen production is shown in F and H of Table I and Fig. 1. The mechanism of this activity undoubtedly involves a degradation of the glucose to intermediary keto acids and the amination of these. Malonate by its blocking of the succinic dehydrogenase system apparently prevented an optimum synthesis of amino nitrogen from glucose and ammonia. A further demonstration of the operation of the tricarboxylic cycle in *H. diminuta* was observed when succinate was included in the reaction medium. In this case significant increases in alanine as well as glutamic acid were observed.

The fate of the ammonia nitrogen in the tapeworm is demonstrated in Fig. 1. It is of interest to note that the major product of incubation, regardless of substrate, was alanine. This may be considered to be an indication of the essentially anaerobic existence of *H. diminuta*. Chromatograms of liver slices following incubation in media fortified by the inclusion of ammonia ions and/or alpha-ketoglutaric acid or pyruvic acid are given for comparison. It should be noted that the marked tendency to concentrate the free amino nitrogen in alanine did not exist here. In contrast to the tapeworm, the glutamic acid-alpha-ketoglutaric acid system appeared

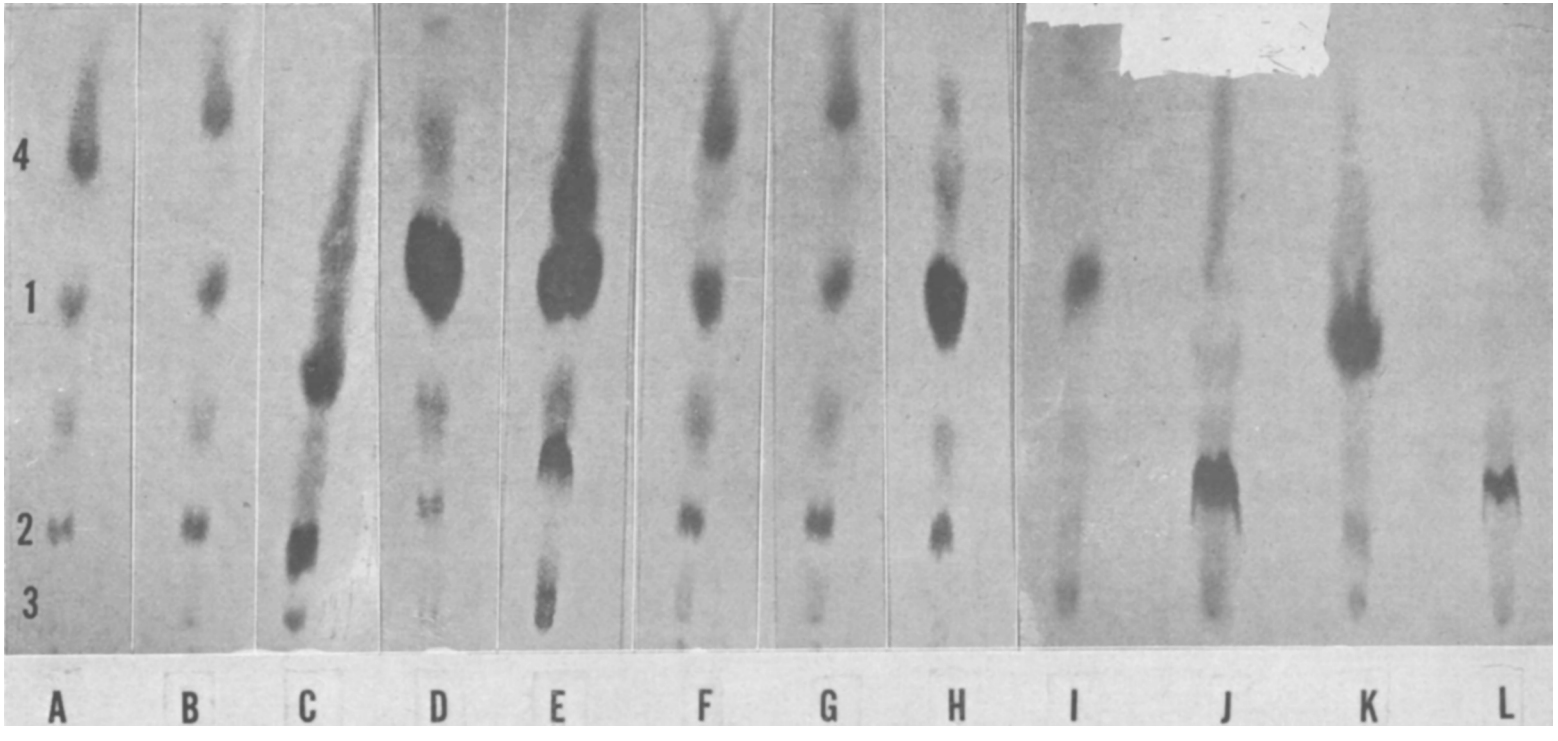

FIG. 1. Paper chromatograms showing amino acids resulting from incubation of *H. diminuta* (A-H) and rat liver slices (I-L) in the following media:

A	KRP	G	KRP + $(NH_4)_2CO_3$ + sodium succinate
B	+ $(NH_4)_2CO_3$	H	+ glucose + sodium malonate
C	+ sodium alpha-ketoglutarate	I	sodium pyruvate
D	+ sodium pyruvate	J	$(NH_4)_2CO_3$ + sodium alpha-ketoglutarate
E	+ sodium oxalacetate	K	+ sodium pyruvate
F	+ glucose	L	sodium alpha-ketoglutarate

1 alanine
2 glutamic acid
3 aspartic acid
4 mixture of amino acids, including methionine, phenylalanine, leucine, and isoleucine

to be dominant, *viz.*, the disappearance of alanine in Exp. 10 and 12 of Fig. 1 in which alpha-ketoglutaric acid was present.

Discussion. The importance of carbohydrates, in addition to their being a primary source of energy, in the metabolism of the rat tapeworm, *H. diminuta,* is indicated by the active participation of the degradation products of glucose in the synthesis of amino nitrogen from ammonia nitrogen. It is of interest to note, in relation to the essentially anaerobic environment of the worm, that the dominant pathway for this activity is through the amination of pyruvic acid to alanine. As has been shown above, this is quite different from the situation that is found in mammalian tissues.

The exact mechanism involved in the incorporation of ammonia nitrogen into the protein molecule is quite uncertain. Some investigators have suggested that this occurs in rat liver by a direct reductive amination of the keto acids(2). Others(7) have recently promoted a more complicated scheme involving the application of the Wood-Werkman condensation of pyruvic acid to oxalacetic acid, the reductive amination of a portion of this, the conversion of the remainder to other keto-acids via the tricarboxylic cycle, and a transamination with certain amino acids. The mechanisms of this synthesis in *H. diminuta* is now under study and it is hoped that information sufficient to describe the process in this organism will be obtained.

Read(8) demonstrated that enzymes catalyzing the oxidation of succinic acid and malic acid were present in homogenates of *H. diminuta.* The data obtained from the current study show that intermediary acids, such as alpha-ketoglutaric and oxalacetic, exist in worm tissues, and that enzymes responsible for their oxidation, and probably that of other acids in the tricarboxylic cycle, function effectively in the oxidation of pyruvic acid in the intact worm. It appears, however, that their activity does not approach the level which is found in such mammalian tissues as rat liver.

It may be observed in Fig. 1 that amino acids other than alanine, glutamic acid and aspartic acid are involved in the total gain in amino nitrogen following incubation with ammonium ions and the respective keto acids. At this time no explanation can be given as to how these other amino acids are related to those that are more directly produced by the incorporation of ammonia nitrogen.

Summary. 1. *H. diminuta,* in the presence of alpha-ketoglutaric acid, pyruvic acid, or oxalacetic acid and ammonium ions is capable of synthesizing amino nitrogen. It appears that this synthesis is so biased that the pyruvic acid-alanine system is favored. 2. Glucose, through its degradation to intermediary keto acids, is also capable of serving as a substrate for the synthesis of amino nitrogen from ammonium nitrogen. The yield, however, does not approach that of the more direct systems. 3. Evidence is given to show that the tricarboxylic cycle actively functions in the living intact worm.

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