3-Hydroxyanthranilic Acid Metabolism V. Distribution of Enzyme System in Animal Tissues.* (19109)

ROBERT E. PRIEST, ANN H. BOKMAN, AND B. S. SCHWEIGERT.

From the Division of Biochemistry and Nutrition, American Meat Institute Foundation and Department of Biochemistry, University of Chicago.

The enzymatic conversion of 3-hydroxyanthranilic acid to quinolinic acid is important from a nutritional standpoint, since it involves intermediate reactions in the metabolism of tryptophan to nicotinic acid. The chemical reactions are unique in that a benzenoid-type compound is transformed to a pyridine-type compound. *In vivo* experiments have shown that tryptophan can replace nicotinic acid in the diets of a variety of animals (rat, chick, dog, pig, horse, man, etc.). It has been shown that rat liver contains the enzyme system that metabolizes 3-hydroxyanthranilic acid to quinolinic acid(1-4) and that complete disappearance of 3-hydroxyanthranilate takes place when incubated with rat kidney slices(5). Other studies(6) have shown that an intermediate compound is formed in the metabolism of 3-hydroxyanthranilic acid to quinolinic acid by acetone-powder preparations or homogenates of rat liver. In order to obtain further information on the enzyme system in rat tissues, and for tissues available in larger quantities than rat liver, a study was made of the distribution of this enzyme system in various tissues of the rat and in pork and beef liver and kidney.

Material and methods. The rat tissues to be tested for the presence of enzyme were obtained from adult albino rats by decapitating the animal, removing the tissues desired, and using them immediately. The beef and pork tissues were frozen within one-half hour after removal from the animal and kept frozen until used. Samples were homogenized as described earlier(3) and diluted appropriately with M/10 phosphate buffer, pH 7.0, centrifuged in a clinical centrifuge at 1700 rpm (Cal. 193G) and the supernatant liquid used as the enzyme source. The day an assay was to be made 3-hydroxyanthranilic acid was dissolved in phosphate buffer, diluted to 50 μg/ml and refrigerated prior to use. After incubating the enzyme preparation and substrate for 30 minutes at 37°C, the enzyme was heat inactivated, the reaction mixture filtered, and aliquots taken for assay. The substrate was assayed fluorimetrically(3), and the product, quinolinic acid, determined microbiologically after decarboxylation to nicotinic acid. *Lactobacillus arabinosus* 17-5 was used as the test organism. Suitable enzyme and substrate blanks were included in each experiment. Rat liver was used as the basis of comparison and the composite data for the enzyme activity of rat, beef, and pork liver are presented in Table I. Comparable data for rat, beef, and pork kidney are shown in Table II. Three or more samples of each tissue with one or more levels of tissue were used in these studies. Acetone-powder preparations(3) were also made of rat and pork liver and beef kidney. These preparations showed the same activity as the homogenates on a dry weight basis.

Results and discussion. Homogenates of rat liver quantitatively converted 3-hydroxyanthranilic acid to quinolinic acid. From

* Supported in part by a grant from the Division of Research Grants and Fellowships, National Institutes of Health, U. S. Public Health Service. We are indebted to Merck and Co. for the 3-hydroxyanthranilic acid used in these studies and to Armour and Co. for their cooperation in obtaining beef and pork liver and kidney. Journal paper No. 43, American Meat Institute Foundation.

1. Schweigert, B. S., *J. Biol. Chem.*, 1949, v178, 707.

2. Schweigert, B. S., and Marquette, M. M., *J. Biol. Chem.*, 1949, v181, 199.

3. Bokman, A. H., and Schweigert, B. S., *Fed. Proc.*, 1950, v9, 152; *J. Biol. Chem.*, 1950, v186, 153.

4. Henderson, L. M., and Ramasarma, G. B., *J. Biol. Chem.*, 1949, v181, 687.

5. Mason, M., and Berg, C. P., *J. Biol. Chem.*, 1951, v188, 783.

6. Bokman, A. H., and Schweigert, B. S., *Fed. Proc.*, 1951, v10, 164, *Arch. Biochem.*, 1951, v33, 270.

TABLE I. Effectiveness of Liver Homogenates in Converting 3-Hydroxyanthranilic Acid to Quinolinic Acid.*

Liver†	Enzyme conc., mg wet wt of tissue	Substrate metabolized, micromoles	Quinolinic acid found, micromoles	% conversion
Rat	10	.37	.39	105
	50	.65	.59	91
Pork	10	.31	.33	106
	50	.61	.52	86
Beef	10	.24	.20	81
	50	.65	.37	56

* .65 micromoles 3-hydroxyanthranilic acid, homogenate and buffer added to a total volume of 10 ml, and incubated at 37° for 30 min.

† Dr. L. M. Henderson and associates (private communication) have also observed that pork and lamb liver are essentially equal in enzyme activity to rat liver in the metabolism of 3-hydroxyanthranilate to quinolinic acid.

TABLE II. Effectiveness of Kidney Homogenates in Converting 3-Hydroxyanthranilic Acid to Quinolinic Acid.*

	Enzyme conc., mg wet wt of tissue	Substrate metabolized, micromoles	Quinolinic acid found, micromoles	% conversion
Rat	10	.21	.15	71
	50	.65	.13	20
	100	.65	.26	40
Beef	20	.35	.24	67
	100	.65	.25	38
Pork	20	.37	.29	78
	100	.64	.33	52

* See footnote, Table I.

91% to 105% of the substrate metabolized by rat liver homogenates and from 75% to 108% of the substrate metabolized by pork liver homogenates could be assayed as quinolinic acid (molar basis). Certainly it can be said that quinolinic acid is the primary product of this enzyme system. While beef liver was equally active in metabolizing 3-hydroxyanthranilic acid, all of the substrate metabolized could not be assayed as quinolinic acid. With the use of 10 mg of tissue about 80% of the substrate metabolized could be accounted for by quinolinic acid assay and with 50 mg of tissue only about 55% was found.

Homogenates of rat, pork, and beef kidney possessed equal activity per unit weight of tissue for metabolizing 3-hydroxyanthranilate (Table II) although only about one-half that observed for liver homogenates. When 10 or 20 mg of tissue were used, a large percentage of the substrate utilized appeared as quinolinic acid, but with 50 or 100 mg of tissue a much smaller percentage conversion was found. The micromoles converted to quinolinic acid remained approximately the same in either case. These results suggest that another primary product of 3-hydroxyanthranilic acid metabolism was formed. Since it is known that an intermediate compound is involved in the conversion of 3-hydroxyanthranilic acid to quinolinic acid(6), it seemed possible that this intermediate had accumulated at the end of the incubation period and could account for the low conversion to quinolinic acid. Kidney homogenates and beef liver homogenates were tested spectrophotometrically(6) for enzyme activity and evidence was obtained that although the intermediate compound was formed with these enzyme preparations, it was also rapidly broken down as shown by a reduction in the optical density at 360 mμ indicating that this intermediate was not an end product of the reaction.

Possibilities to explain these findings include the further transformation of part of the quinolinic acid after it is produced and the metabolism of a portion of the substrate by alternative pathways which may or may not

include the intermediate. An experiment was conducted with the addition of quinolinic acid to beef liver and kidney homogenates and no evidence for the metabolism of the added quinolinic acid was observed.

Several other tissues of the rat were tested and found not to metabolize 3-hydroxyanthranilic acid to quinolinic acid in measurable amounts at the enzyme concentrations used (10-100 mg wet weight of tissue). Muscle, spleen, pancreas, brain and testis homogenates all metabolized small but significant amounts of substrate, but in no case were detectable amounts of quinolinic acid produced, and no spectrophotometric evidence for the formation of the intermediate compound was obtained.

Summary. A survey of various tissues of the rat, and of beef and pork liver and kidney was made for the presence of the enzyme system that converts 3-hydroxyanthranilic acid to quinolinic acid. It was found that rat and pork liver homogenates quantitatively convert 3-hydroxyanthranilic acid to quinolinic acid. Beef liver also contains this enzyme system, but not all of the substrate metabolized could be accounted for as quinolinic acid. Rat, pork and beef kidney likewise contain the enzyme system but again not all of the 3-hydroxyanthranilic acid metabolized was found by the quinolinic acid assay. Spleen, muscle, brain, pancreas and testis of rats either do not contain the enzyme system or contain it in very small quantities. No evidence was obtained to indicate that the intermediate compound previously described had accumulated to account for the lower production of quinolinic acid by these preparations.

Received September 10, 1951. P.S.E.B.M., 1951, v78.

Glucose Formation by Kidneys in Eviscerated Dogs.* (19110)

BRUCE MACKLER, PETER AMMENTORP,† HARRY GRAUBARTH, AND GEORGE M. GUEST.

From the Children's Hospital Research Foundation and the Department of Pediatrics, University of Cincinnati, Cincinnati, O.

Several investigators have offered evidence that the kidneys may be a source of blood-sugar(1-6). Cohn and Kolinsky(6) reported evidence of glucose production by the kidneys, finding the rate of disappearance of glucose from the blood of hepatectomized and nephrectomized dogs nearly double the rate found in dogs hepatectomized without nephrectomy. In experiments here reported, (designed for further study of this question), evisceration was done, with and without nephrectomy, to eliminate possible effects of insulin production on the rate of disappearance of sugar from the blood in hepatectomized dogs.

Procedures. Ten mongrel male and female dogs, previously fasted 3 days with water allowed *ad libitum,* were eviscerated by the method of Markowitz(7) under nembutal anesthesia. Five dogs were bilaterally nephrectomized with the evisceration and in 5 the kidneys were left intact. Samples of venous blood were drawn for analysis immediately following operation and at 15-minute intervals during the next hour. Blood-sugar was de-

* Aided by a grant from the Nutrition Foundation.

† Visiting Research Associate, from Copenhagen, Denmark.

1. Bergman, H., and Drury, D. R., *Am. J. Physiol.,* 1938, v124, 279.

2. Reinecke, R. M., *Am. J. Physiol.,* 1943, v140, 276.

3. Roberts, S., and Samuels, L. T., *Am. J. Physiol,* 1944, v142, 240.

4. Reinecke, R. M., and Hauser, P. J., *Am. J. Physiol.,* 1948, v153, 205.

5. Drury, D. R., Wick, A. N., and MacKay, E. M., *Am. J. Physiol.,* 1950, v163, 655.

6. Cohn, C., and Kolinsky, M., *Am. J. Physiol.,* 1949, v156, 345.

7. Markowitz, J., *Textbook of Experimental Surgery,* William Wood and Company, Baltimore, 1937.