dehalogenation has not been investigated further.

Summary. 1. 5-Iodoorotic acid and 5-iodouridine *per se* are not utilizable for the growth of *S. faecalis* or *L. casei,* while in media supplemented with thymine, thymidine or pteroylglutamic acid, the growth of these organisms is not inhibited by iodoorotic acid or iodouridine. 2. Although 5-iodoorotic acid appeared to support the maximal growth of *L. bulgaricus* 09, evidence was presented that this was attributable to the release of orotic acid from the iodinated derivative. 3. High concentrations of 5-iodoorotic acid inhibited the growth of *L. bulgaricus* 09, but this could not be reversed by orotic acid. This inhibition was attributed to the formation of iodide ion, since potassium iodide in similar concentrations produced a comparable inhibitory effect on the microorganism.

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Enzymatic Hydrolysis of Acyl Naphthylamines.* (20954)

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The literature of enzymes hydrolyzing aryl-substituted acylamides such as acetanilid and phenacetin is very scanty(1-4). In the course of attempts to develop a more accurate quantitation of these enzymes, N-acetyl naphthylamines were tried as substrates because of the high color intensity of dyes obtainable from naphthylamines by azo-coupling. It was soon found that extracts of many organs will hydrolyze acetyl naphthylamines at a low rate. Taking a lead from Birnbaum and coworkers (5), and from Rao and associates(6) who found that chloroacetyl amino acids are hydrolyzed much faster than the corresponding acetyl and glycyl derivatives, chloracetyl naphthylamines were tried next as substrates. They were found to be hydrolyzed by all tissues examined 30 to 100 times faster than the corresponding acetyl compounds. The activity of some tissues was high enough to permit its localization by a histochemical method.

Experimental. Chloroacetyl naphthylamines were prepared by acylating α- and β-naphthylamine with chloroacetyl chloride in an acetone-pyridine medium. The compounds were recrystallized twice from 60% methanol. The corrected melting points are: chloroacetyl α-naphthylamine, 161°C; chloroacetyl β-naphthylamine, 113°C. 0.03 M stock solutions in acetone were prepared of both substances. For use, one ml of the stock solution was blown into 100 ml of 0.04 M buffer. Both the stock solutions and the buffered substrate mixture were found to be stable at room temperature for several weeks. The enzymes used were 1:10 homogenates of fresh human tissues in saline, cleared by the freezing-thawing method(7), and human serum. One ml of enzyme dilution was incubated with 5 ml of buffered substrate for

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TABLE I. Hydrolysis of Chloroacetyl α-naphthylamine by Extract of Human Pancreas. Dependence on pH. Acetate (pH 4.25 to 5.45) or phosphate buffers. 4 mg of tissues/tube. Incubation: 1 hr. Values in percentages of hydrolysis at pH 6.2.

pH	4.25	4.7	5.0	5.45	5.8	6.0	6.2	6.4	6.6	7.15	8.0
% of hydrolysis	19	20	25	41	88	98	100	92	84	57	4

various lengths of time. At the end of incubation the tubes were placed in ice water, and one ml of enzyme was added to the control tubes, incubated without enzyme. One ml of a M acetate buffer of pH 4.2 to 4.5 containing 10% of Tween 20 or 40 was added to the tubes, followed by 0.5 ml of a 0.25 to 0.5% fresh solution of Diazo Red B Salt. The tubes were inverted twice and allowed to stand. They were read between 5 and 15 minutes at 550 mμ against their controls. A curve was standardized with dilutions of naphthylamines containing 2 to 25 μg (±0.02 to 0.2 μ M) of naphthylamine in 5 ml. The reason for using Tween in the solution is its ability to keep the azo dye, utterly insoluble in aqueous media, in a perfectly clear colloidal solution, suitable for direct colorimetry. This principle has been recommended also for other azo dye methods(7).

Results. 1) *pH optimum.* The dependence of the rate of hydrolysis of chloroacetyl α-naphthylamine by human pancreas is shown in Table I. All subsequent experiments were run at pH 6.0 to 6.2.

2) *Rates of hydrolysis of acyl naphthylamines.* Table II shows the rates of hydrolysis of acetyl and chloroacetyl naphthylamines in terms of μg of naphthylamine liberated per hour and per mg of tissue. The much higher rates of hydrolysis of the chloroacetyl compounds is clearly shown; also, the relative preference of the pancreatic enzyme for the β-isomers.

TABLE II. Hydrolysis of Acyl Naphthylamines by Human Tissues. μg of naphthylamine liberated /hr/g of tissue.

	Acetyl α-naphthylamine	Acetyl β-naphthylamine	Cl-acetyl α-naphthylamine	Cl-acetyl β-naphthylamine
Kidney	20	0	640	160
Liver	130	186	4600	1650
Pancreas	13	27	1500	1800

3) *Proportionality between the amount of enzyme and the extent of hydrolysis.* Between 0.01 and 0.15 μM of substrate hydrolyzed in 2 hours, the extent of hydrolysis was proportional to the amount of enzyme used.

4) *Course of hydrolysis in time.* As shown in Table III, during the first few hours of hydrolysis there is a slight gradual inactivation of the enzyme.

TABLE III. Course of Hydrolysis in Time. Human pancreas, 2 mg/tube. μg naphthylamine liberated.

Hr	¼	½	1	2	3	4	5	8	12	24
μg	1.0	2.0	3.5	6.0	8.7	11.3	14	21.2	32.2	63.5

5) *Inhibition effects.* As shown in Table IV, a number of metal ions and taurocholate have a definite inhibitory effect on the enzyme. So far, no activating substances have been found.

6) *Hydrolysis of chloroacetyl α-naphthylamine by human serum.* To date, 30 unselected human sera have been tested for their activity. As a rule, one ml of a 50% dilution of serum was used; length of incubation was 2 hours. Table V shows the activity range of human sera.

7) *The effect of other enzymes.* None of the substrates mentioned were hydrolyzed measurably by the following enzymes: one mg crystalline pepsin (at pH 6.2 and 2.8); one mg crystalline chymotrypsin; 5 mg of crude trypsin or papain; 8 samples of human

TABLE IV. Inhibition Effects. Human liver; 4 mg/tube. 1 hr values.

Addition	None	Ca, 0.01M	Co, 0.01M	Mg, 0.01M	Mn, 0.002M	Taurocholate, 0.004M
μg naphthylamine liberated	18	14.6	10.6	13.2	8.6	5.6

TABLE V. Hydrolysis of Chloroacetyl α-naphthylamine by Human Serum. Range of activity.

μg of naphthylamine liberated /hr/ml serum	0-1.9	2-3.9	4-5.9	6-7.9	8-11
No. of sera	4	13	6	3	4

gastric juice.

8) *The histochemical localization of activity.* When paraffin sections of acetone-fixed tissues were incubated with the chloroacetyl α-naphthylamine substrate mixture in the presence of about 20 mg of Diazo Garnet GBC Salt per Coplin jar (a method analogous to those used for the demonstration of esterases (8), moderately intense but definite localizations were obtained within 30 minutes to 2 hours in some tissues. Owing to the small number of tissues examined, no general statement on the distribution of enzymatic activity can be made at this time. So far, activity could be demonstrated at the following sites: liver and intestinal mucosa of the guinea pig; intestinal mucosa of the rat; bronchial mucosa and convoluted renal tubules of the cat (see Fig. 1 to 3). Human tissues, quite active in the test tube, were invariably inactive in paraffin sections.

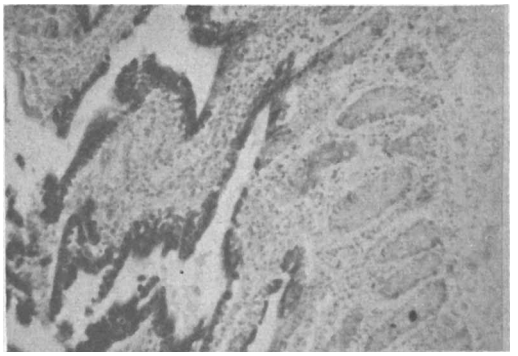

FIG. 1. Intestine of guinea pig. Activity at surface of villi.

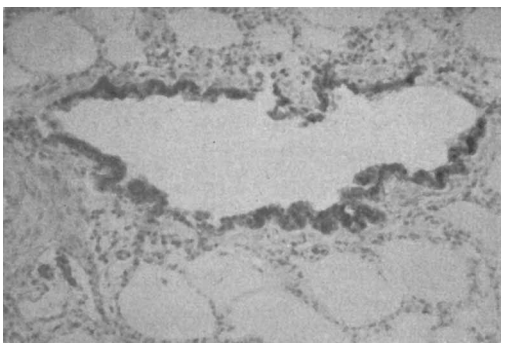

FIG. 2. Lung of cat. Activity in bronchial epithelium.

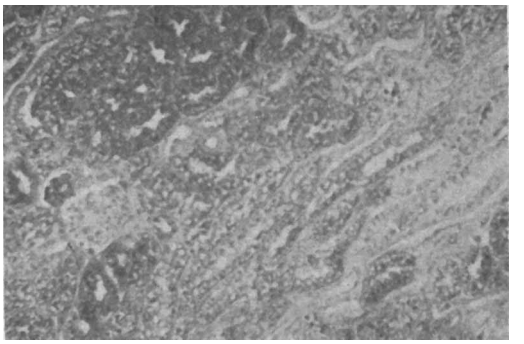

FIG. 3. Kidney of cat. Activity in convoluted tubules.

Conclusions. An enzyme, hydrolyzing acyl naphthylamines, can be demonstrated in a number of tissues and in serum. Chloroacetyl naphthylamines are hydrolyzed at a much higher rate than the corresponding acetyl compounds. It is possible to localize the enzyme histochemically in some tissues.

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