4. For details of the apparatus see H. Leibowitz, N. Myers, and P. Chinetti, *J. Exptl. Psychol.* 50, 15 (1955).
5. C. H. Graham, in *Handbook of Experimental Psychology*, S. S. Stevens, Ed. (Wiley, New York, 1951), Chap. 23; for a review of the literature see also R. S. Woodworth and H. Schlosberg, *Experimental Psychology* (Holt, New York, rev. ed., 1954), Chaps. 15 and 16.

17 October 1955

Studies on Tryptophan and Serotonin in Patients with Malignant Carcinoid

This is a report of an abnormality of tryptophan metabolism in patients with metastatic malignant carcinoid, a relatively rare disease that Thorson *et al.* showed to be associated with an unusual syndrome consisting of intestinal hypermotility, bronchospasm, vasomotor disturbances, and cardiac lesions (*1*). The demonstration of the presence of the pharmacologically active agent, serotonin (5-hydroxytryptamine), in carcinoid tumors (*2*) implicated it in the pathogenesis of this disorder. Since Thorson's report many additional patients with this disease have been reported.

A preliminary report from this laboratory indicated that the urinary excretion of 5-hydroxyindoleacetic acid (5HIAA), the major metabolite of serotonin, was greatly increased in patients with malignant carcinoid (*3*). Since previous animal studies had shown tryptophan to be the dietary precursor of serotonin (*4*), an investigation of tryptophan metabolism was undertaken in patients with this disorder (*5*).

Blood levels of serotonin were found to range from 0.6 to 3.0 μg/ml in carcinoid patients as compared with 0.1 to 0.3 μg/ml in normals. A tremendously elevated excretion of urinary 5HIAA, 70 to 800 mg/day in carcinoid patients as compared with 2 to 9 mg/day in controls, was found to be diagnostic of this condition (*3*).

The excretion of labeled 5HIAA following the administration of 2-C^{14} DL-tryptophan to three of the patients demonstrated that in human beings tryptophan is the precursor of serotonin and its metabolites. A quantitative estimate of the defect in tryptophan metabolism in this disorder was shown in the following experiment. One patient was fed a diet that gave a basic daily intake of 500 mg of tryptophan, and at various intervals the tryptophan intake was increased. The daily excretion of total 5-hydroxyindoles and of 5HIAA was measured and nitrogen balance was determined, as shown in Fig. 1. The urinary excretion of 5-hydroxyindoles increased when the tryptophan intake was increased and returned to control levels when the original intake was resumed. At a daily intake of 500 mg, as much as 60 percent of the dietary trytophan was converted to 5-hydroxyindoles. In normals only about 1 percent is metabolized in this way. The normal requirement of tryptophan has been estimated by Rose to be 150 to 200 mg/day (*6*). If this is correct, then this patient was just maintained in nitrogen balance at an intake of 500 mg.

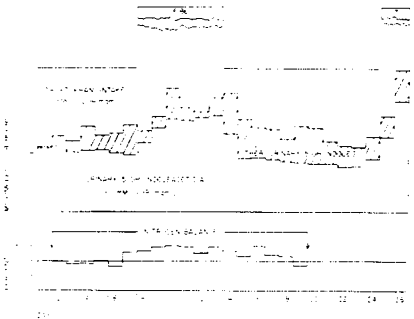

Fig. 1. The effect of variations in tryptophan intake on nitrogen balance and urinary excretion of 5-hydroxyindole compounds.

It is apparent that the carcinoid tumor is parasitic upon the tryptophan stores of the patient and, as a result, less of this amino acid may be available for formation of other metabolites, such as protein and niacin. Weight loss and hypoproteinemia are common features of the condition, and pellagra has been reported in several cases. The complex manifestations of this disorder may be related to both a serotonin excess and a tryptophan deficiency.

The conversion of what is normally a minor pathway of tryptophan metabolism into a predominant route of metabolism may make patients with malignant carcinoid useful in further studies on the biochemistry and physiology of serotonin.

Details of these studies will be published elsewhere (*7*).

SIDNEY UDENFRIEND
HERBERT WEISSBACH
ALBERT SJOERDSMA

National Heart Institute,
National Institutes of Health,
Bethesda, Maryland

References and Notes

1. A. Thorson *et al.*, *Am. Heart J.* 47, 795 (1954).
2. F. Lembeck, *Nature* 172, 910 (1953).
3. A. Sjoerdsma, H. Weissbach, S. Udenfriend, *J. Am. Med. Assoc.* 159, 397 (1955).
4. S. Udenfriend and E. O. Titus, *Amino Acid Metabolism* (Johns Hopkins Press, Baltimore, 1955).
5. Five patients with malignant carcinoid were studied at the National Heart Institute. Urine specimens were obtained from five additional patients at other institutions.
6. W. C. Rose, G. E. Lambert, M. J. Coon, *J. Biol. Chem.* 211, 815 (1954).
7. A. Sjoerdsma, H. Weissbach, S. Udenfriend, *Am. J. Med.*, in press.

19 October 1955

Elastase Production in the Canine Pancreas

In 1950 Balo and Banga (*1*) reported the action of an extract of beef pancreas on pure elastin. The active substance, in view of its specificity, was termed "elastase." This work has been confirmed many times, and in addition these authors have reported that in human atherosclerosis the quantity of elastase extractable from the pancreas diminishes (*2*). Pepler and Brandt (*3*) demonstrated the action of elastase on the ground substance of aorta; the possibility of this enzyme's playing a part in the development of atherosclerosis has been noted further recently (*4*).

The following observations have been made concerning the production of elastase in dog pancreas. Pancreatic function in healthy mongrel dogs was modified by the following procedures.

1) Alloxan destruction of beta cells in the pancreatic islets. Two doses of alloxan (75 mg/kg. body weight) were given intravenously at 24-hour intervals. The animals were sacrificed 24 hours after the last dose. The loss of granules in the beta cells was demonstrated histologically.

2) Destruction of both alpha and beta cells in the pancreatic islets. Two doses of alloxan were given as before; following the last dose, 300 mg of cobalt chloride in 25 ml of water were given intravenously at hourly intervals for three doses. The animals were sacrificed 1 hour after the last dose. Complete lysis of both alpha and beta cells was demonstrated histologically.

3) Destruction of the pancreatic acinar tissue. Progressive destruction by fibrosis was produced by gradual occlusion of the pancreatic duct by a magnesium band placed about the duct. The technique, described elsewhere (*5*), provides both fibrosed and normal pancreatic tissue for assay in the same animal.

Elastase assays of residual pancreatic tissue were made by the method of Hall and Gardiner (*6*), over an incubation time of 18 to 20 hours, after preparation of the extracts by the method of Balo and Banga (*1*).

The elastolytic activity of pancreatic extracts varies a little with the elastin preparation and the results shown here in group 1 and groups 2a and 2b represent experiments carried out on three separate preparations of elastin.

Group 1a (control). Thirty-one assays of normal pancreatic tissue had an average elastolytic activity of 0.51 mg of elastin per hour (S.D. = 0.12).

Group 1b (experiment). Sixteen assays of pancreatic tissue modified by the administration of alloxan (method 1) showed an average elastolytic activity of 0.31 mg per hour (S.D. = 0.112).

The standard error of the difference between the two groups is 0.12, and it is considered that destruction of the beta cells in the islets of dog pancreas does not significantly alter the rate of production of elastase.

Group 1c (experiment). Twenty-four assays of pancreatic tissue in which both alpha and beta cells had been destroyed (method 2) showed no elastolytic activity, suggesting that destruction of the alpha cells is responsible for the failure of elastase production.

Group 2a (control). Seven assays of normal pancreatic tissue provided by experimental method 3 showed an average elastolytic activity of 0.36 mg per hour (S.D. = 0.12).

Group 2a (experiment). Seven assays of fibrosed pancreatic tissue from the same experiments showed no elastolytic activity, against the same elastin preparation.

Group 2b (control). Fifteen assays of normal pancreatic tissue showed an average elastolytic activity of 0.24 mg per hour (S.D. = 0.06).

Group 2b (experiment). Fifteen assays of fibrosed pancreatic tissue showed no activity against the same elastin preparation as in the control.

It appears, as described by Lansing, Rosenthal, and Alex (7) in teleost fishes, that the dog pancreas produces its elastase in the alpha cells of the islets. If the observation (2) that human atherosclerosis is associated with lowered elastase production by the pancreas is confirmed, it may be that atherosclerosis is a function of alpha islet cell failure.

A. E. CARTER*

Department of Surgical Research, Harvard University, and Peter Bent Brigham Hospital, Boston, Massachusetts

References and Notes

1. I. Balo and J. Banga, *Biochem. J. London* 46, 384 (1950).
2. ———, *Acta Physiol. Acad. Sci. Hung.* 4, 187 (1952).
3. W. J. Pepler and F. A. Brandt, *Brit. J. Exptl. Pathol.* 35, 41 (1954).
4. "Atherosclerosis," *Natl. Acad. Sci.–Natl. Research Council Publ. No. 338* (1955).
5. A. E. Carter, in preparation.
6. D. A. Hall and J. E. Gardiner, *Biochem. J. London* 59, 465 (1955).
7. A. I. Lansing, T. B. Rosenthal, M. Alex, *Proc. Soc. Exptl. Biol. Med.* 84, 689 (1953).

* Present address: 10, Elgar Ave., London W.5.

26 September 1955

Occurrence of 2-Hexenal in the Cockroach Eurycotis floridana

Eurycotis floridana (Walker), a large wingless cockroach, has been recorded in Georgia, Florida, and Mississippi, occurring in outdoor sheltered areas such as stumps, under signs, and the bark of dead trees (*1*). When alarmed, the adults, but not the nymphs, emit a secretion that has an odor which Hebard (*1*) has likened to that given off by the hemipteron, *Brochymena annulata* (Fabricius).

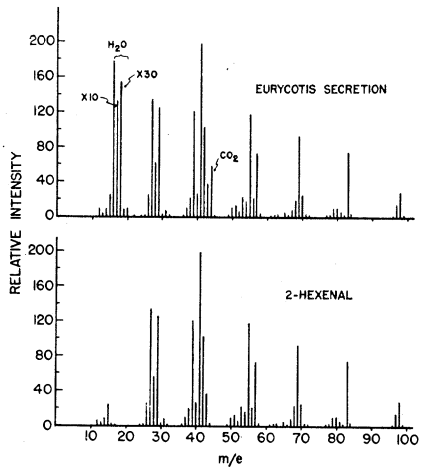

Fig. 1. Comparison of the mass spectra of 2-hexenal and the *Eurycotis* secretion.

In *Eurycotis,* the chemical is secreted by glandular cells into a large bilobed sac, where it is stored as a yellow liquid. The sac opens to the outside of the body medially in the intersegmental membrane between the sixth and seventh abdominal sternites. The location of this gland is similar to that found in *Blatta orientalis* (*2*).

When ejected, the volatile secretion from *Eurycotis* issues as a fine spray or as droplets that may be thrown for a distance of several inches. The secretion is toxic to the cockroaches if the insects are confined without suitable ventilation and made to emit the material. A similar toxicity occurs with the beetles *Tribolium* which are killed by their own odorous secretions (*3*), consisting mainly of ethylquinone (*4*). The *Eurycotis* secretion may be irritating if it gets on sensitive skin areas.

By means of mass spectrometry and infrared spectrophotometry, and the preparation of a chemical derivative, the *Eurycotis* secretion has been identified as 2-hexenal (*trans*). The sample, first analyzed in a Consolidated 21-103B analytic mass spectrometer, was prepared by simply dissecting out the reservoirs from adult males and females; some fatty tissue and parts of the sternites were also included. The sample was placed in a small tube that was attached directly to the inlet system of the mass spectrometer. The tube was cooled to – 180°C to condense the volatile components present, and then it was evacuated. The coolant was removed from the tube, and the condensed material was volatilized directly into the inlet system of the mass spectrometer. The first fraction collected at a pressure of 100 μ (volume of inlet system was 3 lit) was analyzed, as were a middle cut and the material present in the system at room temperature.

Structural analysis of the fragmentation pattern of the secretion showed that the material is a hexenal. Comparison of the spectrum of the secretion with the spectrum of various hexenals identified the secretion as pure 2-hexenal (Fig. 1). The secretion was contaminated only with CO_2 and H_2O, which condensed during the initial freezing from the air and from other tissues that were dissected with the glands. No trace of other impurities could be found. The CO_2 and H_2O may be mathematically removed from the spectrum of the *Eurycotis* secretion; the residue has a spectrum identical with that obtained from 2-hexenal. The spectra of the glandular secretions from male and female *Eurycotis* were identical.

The sample for infrared analysis was obtained by holding several males and allowing them to eject their secretion into carbon tetrachloride. The curve obtained was similar to that of synthetic 2-hexenal (Fig. 2) and further indicated the secretion is the *trans* form of the compound (*5*).

The 2,4-dinitrophenylhydrazone was prepared by allowing the cockroaches to eject their secretion into freshly prepared reagent (*5*). The melting point of the derivative was 139.5°–141.5°C, uncorrected (compare mp of 2,4-dinitrophenylhydrazone, 141°–142°C, *6*).

2-Hexenal was found to be one of the several carbonyl compounds responsible for the odor of whale oil (*6*). Other natural sources of this aldehyde are found in the plant kingdom. It is one of the constituents that make up the odor of Java citronella (*7*) and lavender oil (*8*). It forms part of the flavor of green tea (*9*), and the aroma of tea is primarily this compound (*10*). It has also been isolated from mulberry leaves (*11*).

Although nothing is known of the ene-

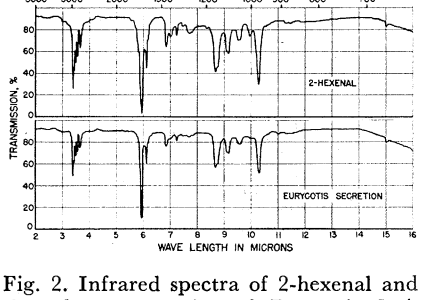

Fig. 2. Infrared spectra of 2-hexenal and the odorous secretion of *Eurycotis floridana.* Baird spectrograph with NaCl prism; samples in carbon tetrachloride solution; cell thickness, 0.1 mm and 0.418 mm, respectively.