of drug per cell appears to be critical, small variations in cell number and drug concentration may produce relatively large effects. The effects of the drugs on the assimilation, however, were consistent. Oxygen uptake measurements were made in Warburg vessels containing 2.0 ml fluid volume. Ammonia determinations were made on the supernate of cells which had been centrifuged down after the addition of 0.2 ml 20% trichloroacetic acid. After suitable dilution, Nessler's reagent was added and the color read at 420 mμ.

The Table shows that the addition of potassium is necessary for effective ammonia assimilation as shown by the ratio of ammonium ions assimilated to oxygen taken up. This ratio is not affected by the time of the incubation until the substrate concentration becomes limiting. The potassium requirement may be the result of potassium loss which occurs when the cells are washed in water. In 40 ml of wash water there were on the average 5.7 m*M* of potassium. The estimations were made with the flame photometer. It also shows that polymyxin B and benzalkonium chloride can restore ammonia assimilation to a significant degree. That the anionic drug is less effective suggests that the elimination of a barrier to ammonium ions is not the only mechanism of increased assimilation but that the cationic drugs may be substituting for potassium in this reaction. When the drugs are added in this or lower concentrations to the cells in the presence of potassium, they are without effect.

Riassunto. Cellule di *Pseudomonas aeruginosa* perdono potassio durante lavaggio in acqua e contemporaneamente esibiscono bassi livelli di utilizzazione di ioni ammonio in presenza di substrato ossidabile. Un rilevante aumento di utilizzazione di ammonio è indotto dall'aggiunta di potassio o detergenti cationici (polimixina B, cloruro di benzalkonio), ma non dall'aggiunta di un detergente anionico.

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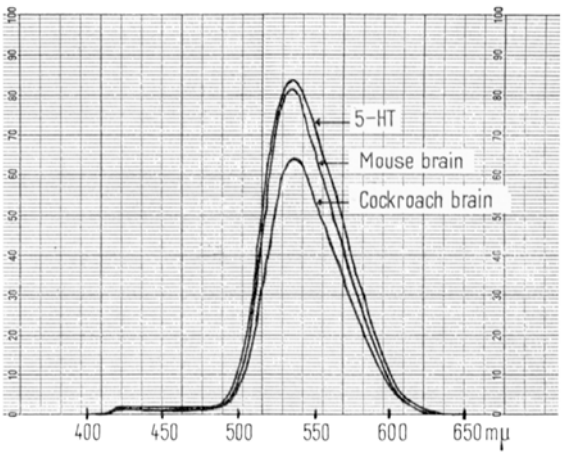
Synthesis of 5-Hydroxytryptamine in the American Cockroach

In insects venom glands 5-hydroxytryptamine (5-HT) has been identified with some degree of certainty (Jaques and Schachter[1], Erspamer[2], Bhoola et al.[3]). The amounts of the compound are much in excess of the values reported by Welsh and Moorhead[4] for ground up bodies of several insect species. Davey[5] indicated that a gland (utriculi majores) of the reproductive system of the male American cockroach contained an *o*-dihydroxyindolealkylamine. The properties of the substance would appear to be similar to those of a compound found in pericardial organs of Crustacea (Carlisle[6]), and subsequently stated to be 5,6-dihydroxytryptamine (5.6-diHT) by Carlisle and Knowles[7]. However, Gersch et al.[8] believe that nervous tissue of the cockroach contains 5-HT. Colhoun and Blaschko (in manuscript) have shown that 5,6-diHT was almost as pharmacologically active as 5-HT when tested upon the rat fundus muscle preparation. Unpublished observations have shown that both compounds at 10^{-8} to $10^{-9}M$ increased the rate of contraction of fore and hind gut, movement of malpighian tubules and rate of heart beat of the American cockroach. Bromolysergic acid diethylamide was an efective blocking agent. Although of pharmacological interest these observations are of little importance in the physiology of the cockroach until the distribution and identity of endogenous indole compounds is established.

A large number of tissues of the cockroach were subjected to extraction procedures for differential bioassay, ultraviolet absorption and spectrophotofluorometer techniques for the identification of indole compounds. Briefly, the results show the presence of a substance with the characteristics of 5-HT in brain, corpora cardiaca glands and ventral nerve cord. On a tissue weight basis the highest amount was found in glands, then brain and ventral nerve cord. Because of loss of active substance during the overall extraction procedure for chromatography and subsequent bioassay, the actual amount of 5-HT in tissues has not been determined. The amounts are extremely low in comparison with the titre of acetylcholine in brain and ventral nerve cord. Indeed the small quantity of 5-HT suggests localized distribution in nervous tissue.

Results with other tissues such as gut, malpighian tubules and utriculi majores have been inconclusive. In comparison with data for neural tissue it would seem possible that these tissues contain a substance not identified as 5-HT, or, equally important, 5,6-diHT.



Fluorescence spectra in 3*N* HCl of synthesized 5-HT from mouse and cockroach brain. Excitation monochromator set at 300–305 mμ.

[1] R. Jaques and M. Schachter, Brit. J. Pharmacol. *9*, 53 (1954).
[2] V. Erspamer, Pharmacol. Rev. *6*, 425 (1954).
[3] K. D. Bhoola, J. Calle, and M. Schachter, J. Physiol. *151*, 35 P (1960).
[4] J. Welsh and M. Moorhead, J. Neurochem. *6*, 146 (1960).
[5] K. G. Davey, Can. J. Zool. *38*, 39 (1960).
[6] D. B. Carlisle, Biochem. J. *63*, 32 P (1956).
[7] D. B. Carlisle and F. Knowles, *Endocrine Control in Crustacea* (Cambridge University Press 1959), p. 72.
[8] M. Gersch, F. Fischer, H. Unger, and W. Kabitzer, Z. Naturf. *16b*, 351 (1961).

In a search for other means of identifying the small amount of 5-HT substance in the cockroach, attempts were made to synthesize 5-HT from 5-hydroxytryptophan. Tissues were homogenized in 0.25 *M* sucrose and they were incubated anaerobically at 37.5°C in the presence of substrates according to the technique of KUNTZMAN et al.[9]. Mouse brain extracts were used for comparative purposes. The spectrophotofluorometric results illustrated in Figure 1 show the presence of a substance similar to 5-HT formed by extracts of roach and mouse brain. The rate of decarboxylation in extracts of the insect brain was equal to if not greater than that of mouse brain. About 300 µg of 5-HT was formed by extract of brain in 1 h. This value is qualitative as it was determined following purification procedures and no attempt has been made to correct for losses. A lower rate of synthesis of 5-HT was found with extracts of ventral nerve cord. The identity of 5-HT was confirmed by use of chromatography and the rat fundus muscle preparation with bromolysergic acid diethylamide as a blocking agent. The synthesized 5-HT was active when tested upon the heart and gut of the cockroach. It is concluded that neural tissue of the cockroach contains a decarboxylase forming a substance identified as 5-HT, when 5-hydroxytryptophan is used as a precursor. These results are in agreement with the identification of the endogenous indole compound found in neural tissue. Using maximum concentrations of visceral tissue and utriculi majores glands as sources of enzyme, no decarboxylation of 5-hydroxytryptophan was detected.

Do these tissues contain an indole compound with potent biological activity and differing in chemical structure from 5-HT? WELSH and MOORHEAD[4] identified 5-HT in pericardial organs of crab. Turning to the findings of CARLISLE[6] it would seem possible that pericardial organs also contain 5,6-diHT. However, the evidence of CARLISLE[6] is based upon enzyme oxidation and chromatography whereas that of WELSH and MOORHEAD[4] depends upon chromatography and spectrophotofluorometer assay. In the present work a source of 5,6-diHT made it possible to compare properties of 5-HT and 5,6-diHT with substances extracted from insect tissue. In the same solvent system for chromatography the more polar dihydroxy compound had a lower Rf than 5-HT. Furthermore the ultra violet absorption properties differed markedly. Thus there is no difficulty in separating the two compounds by chemical methods. The tissues of the cockroaches examined gave no evidence of the presence of 5,6-diHT, although caution must be exercised in the extraction of this substance from tissues, for it is easily oxidized in the alkaline extraction medium unless oxygen is excluded. DAVEY[5] used enzymes in the manner of CARLISLE[6] to characterize the indole compound in utriculi majores of the cockroach. His result was analogous to that of CARLISLE[6]. Perhaps the utriculi majores of the cockroach contain a substance oxidized by amine oxidase or an *o*-diphenolase which is not 5-HT or 5,6-diHT[10].

Zusammenfassung. Biologischer und chemischer Nachweis von 5-Hydroxytryptamin im Nervengewebe von *Periplaneta americana* und erster Bericht über die Synthese: Die Substanz konnte in Geschlechtsdrüsen, Eingeweiden und Malpighischen Gefässen nicht positiv identifiziert werden. Sie wird bei enthirnten und bauchmarklosen Tieren zu 5-Hydroxytryptamin decarboxyliert.

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The Research Institute, Canada Department of Agriculture, London (Ontario, Canada), June 21, 1962.

[9] R. KUNTZMAN, P. A. SHORE, D. BOGDANSKI, and B. B. BRODIE, J. Neurochem. *6*, 226 (1961).

[10] The technical assistance of Miss JUDY ELLIOT is gratefully acknowledged.

Demonstration of Anti-Hydralazine Antibody in Hydralazine Induced *Lupus erythematosus*

An increasing number of commonly used drugs have become associated with the apparent induction of a syndrome similar to systemic *Lupus erythematosus* (SLE)[1]. Included among these is the anti-hypertension drug hydralazine (Apresoline®, Ciba Pharmaceutical Co.; 1-hydrazinophthalazine), which, since 1954, has been implicated by a number of workers as the cause of 'hydralazine Lupus syndrome' in approximately 10% of those individuals treated with large doses of the drug (200–800 mg daily) for long periods of time[2]. The fact that idiopathic SLE is associated with various 'abnormal' antibodies (L.E. cell factor, anti-nucleoprotein antibodies, anti-γ-globulin antibody, and the like) prompted a search in this laboratory for a possible anti-hydralazine-antibody in an individual who presented clinical and laboratory evidence of hydralazine Lupus syndrome.

The subject, a 63-year old negro female, had been treated for long term hypertension with hydralazine at a dosage of 200–400 mg/day for 3 years prior to study. Relatively severe clinical symptoms of hydralazine induced SLE were markedly reduced by withdrawal of the drug. Serum specimens were obtained two weeks following admission to the hospital (upon diagnosis of the syndrome) and ten months thereafter. Both specimens were positive for the L.E. cell factor, and were negative by latex agglutination tests for anti-nucleoprotein, anti-thyroglobulin, and anti-γ-globulin antibodies. This report is concerned with the laboratory detection of another 'antibody factor' in this patient's serum, and the induction of a similar factor in several rabbits by long term 'immunization' with hydralazine emulsified in adjuvants.

Though there were no detectable *in vitro* reactions between the patient's serum samples and hydralazine as tested by precipitin and complement fixation techniques, it was found that the patient's serum specimens would agglutinate suspensions of rabbit or sheep red blood cells conjugated with hydralazine hydrochloride by means of the biz-diazotized benzidine (BDB) technique[3]. Such conjugated cells were prepared by reacting a 2.5% suspension of washed erythrocytes with an 0.1% solution of hydralazine hydrochloride (Ciba) in pH 7.2 phosphate buffer and a 1:15 dilution of BDB. The serum specimens were

[1] H. L. HOLLEY, Ann. int. Med. *55*, 1036 (1961).

[2] P. COMENS, *Inflammation and Diseases of Connective Tissue, a Hahnemann Symposium* (W. B. Saunders Co., Philadelphia 1961).

[3] A. B. STAVITSKY and E. R. ARQUILLA, Int. Arch. Allergy *13*, 1 (1958).