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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[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).

heated at 56°C for 30 min and absorbed with equal volumes of non-treated erythrocytes for 30 min at room temperature. To serial dilutions of the serum samples (prepared in 0.5 ml volumes in phosphate buffer—1:100 normal rabbit serum diluent) were added 0.05 ml volumes of the hydralazine-red blood cell conjugates. The initial serum specimen (2 weeks) consistently reacted with a titer of 1:240, while the second serum specimen (10 months) had a titer of 1:80 (Table). These titers could be completely inhibited by prior incubation of the sera with free hydralazine hydrochloride (1.0 mg to 10.0 mg) prior to addition of the conjugated red cells, but not by prior addition of bovine serum albumin (1 mg/ml), ragweed pollen extract (0.1 mg/ml) or penicillin (1000 u/ml). Similarly, the serum samples did not agglutinate red cell conjugated by means of BDB to bovine serum albumin, ragweed extract, or penicillin. Several sets of 'control' sera were tested with the hydralazine-red cell preparations as follows: (a) three serum specimens from clinically diagnosed cases of SLE (ranging from 1 to 5 years duration and presumably unrelated to hydralazine therapy) were negative for hemagglutination activity; (b) 27 serum samples, from the clinical serology laboratory obtained from presumably normal blood donors, were negative for anti-hydralazine hemagglutination activity. On the other hand, sera from 4 rabbits, following a series of multiple injections over a period of 6 months with hydralazine (10 mg hydralazine/ml Freund's adjuvant as a water-in-oil emulsion) gave positive hemagglutination reactions with hydralazine-red blood cell suspensions. Pre-injection and early post-injection rabbit sera were negative. Late post injection sera from two rabbits (six months after the initial series of injections) had titers of 1:80, while sera of the other two rabbits gave a titer of 1:20. These titers could be completely inhibited by prior incubation of the sera with hydralazine, but not with the other antigens used above.

Comparative inhibition of hemagglutination of patient's and 'immune' rabbit sera and hydralazine-RBC suspension by prior incubation with buffer, free hydralazine or other antigens

| Preincubation | Hemagglutination titer | | | | | |
|---|---|---|---|---|---|---|
| | Patient's serum | | Rabbit sera (*post*) | | | |
| | A (2 week) | B (10 month) | a | b | c | d |
| None (buffer) | 1:240 | 1:80 | 1:80 | 1:80 | 1:20 | 1:20 |
| Hydralazine 0.1 mg | 1:40 | 1:20 | 1:20 | 1:15 | 1:2 | 1:2 |
| Hydralazine 1.0 mg | 1:10 | 1:2 | 1:4 | 1:2 | 0 | 0 |
| Hydralazine 10.0 mg | 0 | 0 | 0 | 0 | 0 | 0 |
| Ragweed Pollen extract 7500 PNU | 1:240 | 1:80 | 1:80 | 1:80 | 1:20 | 1:20 |
| Bovine serum albumin 10 mg | 1:240 | 1:80 | 1:80 | 1:80 | 1:20 | 1:20 |
| Penicillin 10000 U | 1:240 | 1:80 | 1:80 | 1:80 | 1:20 | 1:20 |

The anti-hydralazine activity of the reactive sera, both from the subject and the rabbits, was associated with $\gamma$-globulin. Serum samples were subjected to zone electrophoresis in 1% agar. Following electrophoresis at 150 volts for 4 h, 0.5 cm agar strips were eluted into phosphate buffer. Anti-hydralazine hemagglutination activity was recovered only in the eluates corresponding to the $\gamma$-globulin region, but not in eluates identified by paper electrophoresis or immunophoresis as containing albumin, $\alpha$- or $\beta$-globulins. The anti-hydralazine activity of the $\gamma$-globulin eluates could be eliminated by prior reaction of the eluate with appropriate goat anti-$\gamma$-globulin sera for 1 h at 37°C.

These observations suggest that an anti-hydralazine factor, presumably antibody, may be found associated with SLE-like symptomatology following long term therapy with the drug. A similar factor can be obtained after long term injection of rabbits with hydralazine. Others have observed a possible SLE like symptomatology (L.E. cell factor, suggestive tissue pathology, etc.) in several experimental animal species following feeding of large quantities of hydralazine over long periods of time[4,5]. It is not known whether such symptomatology, either serological or histological, is causal or incidental. It is presently premature to postulate any immunological relationship between hydralazine, the anti-hydralazine factor described here, and the hydralazine Lupus syndrome or idiopathic SLE. On the other hand, it would be of value to examine anti-hydralazine antibodies in as many individuals as possible who present a similar hydralazine Lupus syndrome. Similarly, it should be of interest to study sera from other individuals who have taken the drug in various quantities, but who have not had the syndrome, as well as sera from more patients with SLE unrelated to hydralazine therapy.

*Résumé.* La présence d'un facteur anti-hydralazine, probablement un anticorps, a été démontrée par l'emploi d'une technique d'hémagglutination dans le sérum d'un malade qui manifestait des symptômes de lupus érythémateux après un long traitement avec de l'hydralazine. Le même facteur anti-hydralazine a été observé dans le sérum des lapins qui avaient reçu des injections répétées d'émulsion d'hydralazine dans un adjuvant.

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[4] P. Comens, J. lab. clin. Med. *47*, 444 (1956).
[5] F. Siquier, C. Betourne, and J. Bonnet de la Tour, Sem. Hosp. *34*, 773 (1958).

## Über die PAS-Glykogen-Reaktion im Bereich der Ringbinden bei *Amblystoma mexicanum* Cope

Hinweise auf das Auftreten, die Bedeutung und das Schrifttum der Ringbinden brachten bereits frühere Notizen[1-3]. Bisher sind an diesen Strukturen histochemische Untersuchungen kaum ausgeführt worden und zwar vorallem aus zweierlei Gründen: 1. wegen ihres unsteten und seltenen Auftretens und 2. deswegen, weil histochemische Arbeitsweisen die morphologischen Gegebenheiten so verunstalten, dass ihre Erkennung schwierig werden kann. Da es aber nicht klar ist, ob die Ringbinden normale oder pathologische Gebilde sind, erscheint ihre histochemische Bearbeitung sinnvoll.

[1] A. Jonecko, Folia morphol. (Warschau) *9*, 143 (1958).
[2] A. Jonecko, Inaug.-Diss., Zabrze-Rokitnica (1961).
[3] A. Jonecko, Exper. *18*, 166 (1962).