White rats of 1.5–3 months were injected intraperitoneally with 5 mg/kg of reserpine and sacrificed 10 min to 8 days thereafter. Another group was injected with a similar dosis during four consecutive days and the rats were sacrificed 1 h after the last injection. The preparative techniques for the electron microscope were those previously described[2].

On the electron micrographs, the morphological aspect of the plurivesicular component was studied in the treated animal and compared with that of parallel normal controls. Measurements of the diameter of the vesicles and granules and determinations of size distribution were carried out in the controls and 2 h after reserpine injection (Fig. 3). The relative proportion of homogeneous to heterogeneous vesicles was determined in a total of 500 elements for each particular experiment (Fig. 4).

It was found that reserpine produces the almost complete disappearance of heterogeneous vesicles containing dense granules between 2 and 48 h after a single injection

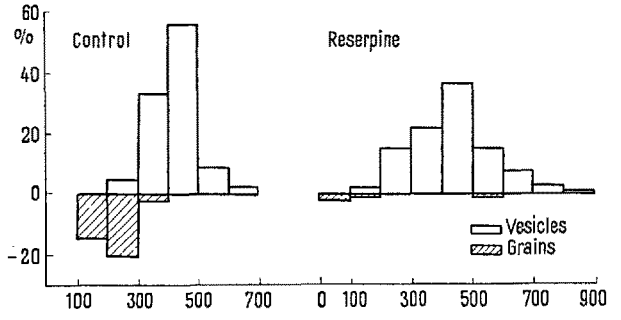

Fig. 3. Histograms of sizes of vesicles in the secretory processes of the control rat and 2 h after injection of reserpine. In the upper part distribution of sizes of 100 vesicles, below distribution of sizes of dense grains/100 vesicles. See the almost disappearance of grains with reserpine (only 22 grains in 600 vesicles).

(compare Fig. 1 and 2). There is a decrease in size of all vesicles (Fig. 3) and some of them resemble sections of the tubular elements found in the pedicle of the expansion[2]. The changes in the relative proportion of the two types of vesicles demonstrate that the drug has an action in as early as 10 min, reaching the maximal effect 2 h after the injection (Fig. 2).

The storage of dense granules was very small after 24 and 48 h increasing notably after 3 days and reaching an almost normal content after the 6th and 8th day. The curve of grain restoration is strikingly similar to that obtained by SHORE and BRODIE[10] with the recovery of serotonin and noradrenaline in the brain of rabbits after injection with reserpine (Fig. 4).

Chronic administration of reserpine leads to the almost complete disappearance of the dense granules and to other profound changes in the vesicular components of the pinealocytic expansion.

All these findings suggest that the secretory processes of the pinealocyte contain the biogenic amines and that these are localized in the plurivesicular material. The fact that reserpine produces the release of serotonin, noradrenaline and dopamine and that these amines may reduce osmium tetroxide, makes a finer morphological discrimination difficult at the present time.

Further pharmacological studies with other drugs that may influence the metabolism of biogenic amines should be carried out. The observations made so far demonstrate that the electron microscope may be a useful tool for studying the sites of storage and mechanisms of release of biogenic amines in the pineal gland.

Zusammenfassung. Pinealdrüsen von Ratten nach Reserpin-Injektion wurden elektronenmikroskopisch untersucht. Die Behandlung ergab auffallende Veränderungen in den plurivaskulären Sekretionsprozessen[2]. Die dichten Bläschen verschwanden innerhalb 2–48 h nach der Reserpingabe nahezu vollständig und erschienen erst wieder zwischen dem 3. bis 8. Tag. Dieser Wechsel verläuft parallel zum Wiederauftreten von Serotonin und Noradrenalin nach SHORE und BRODIE[10].

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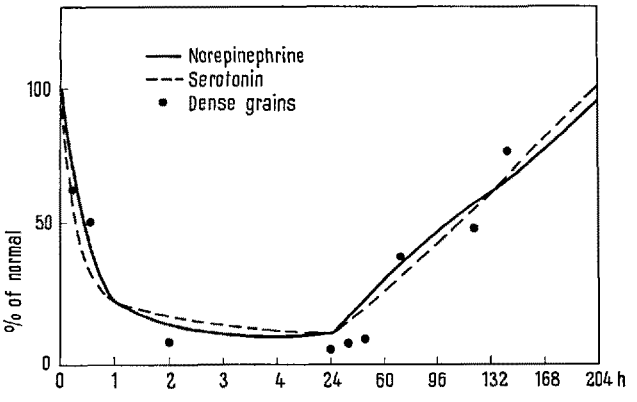


Fig. 4. Curve of SHORE and BRODIE[10] showing the concentration of serotonin and noradrenaline (in % of the normal) in the nervous tissue after a single injection of reserpine. On this curve the points indicate the % change of the normal in dense granules found in our experiments. (100% represent the number of dense grains/100 vesicles found in the normal controls).

[10] P. A. SHORE and B. B. BRODIE, in *Psychotropic Drugs* (Ed. S. Garattini and V. Ghetti, Milano 1957), p. 423.

Tryptophan, Precursor of Tropic Acid in *Datura Stramonium*

Hyoscyamine is the ester of tropine and *l*-tropic acid. Considerable work has been done on the biogenesis of this alkaloid[1]. The pyrrolidine part of tropine has been shown to derive from ornithine[2], but the origin of the residual portion of it has not yet been established[3]. Likewise, the biogenesis of the tropic acid moiety of hyoscyamine has remained in darkness.

Tropic acid – α-phenyl-β-hydroxypropionic acid – has been variously suggested to be of terpenoid origin[4] or to be formed from prephenic acid[5]. LEETE has shown re-

[1] W. O. JAMES, *The Alkaloids*, Vol. 1 (R. H. F. MANSKE and H. L. HOLMES, Academic Press Inc., N.Y. 1950), p. 64.
[2] E. LEETE, L. MARION, and J. D. SPENSER, Can. J. Chem. *32*, 1116 (1954).
[3] A. V. ROBERTSON and L. MARION, Can. J. Chem. *38*, 294 (1960).
[4] E. M. TRAUTNER, Austr. Chem. Inst. J. and Proc. *14*, 411 (1947).
[5] E. WENKERT, Exper. *15*, 165 (1959).

	Activities in counts/min (corrected for background)
Carbon dioxide	2250
Styrene fraction	17

cently[6] that phenylalanine-3-C^{14} gives rise, in *Datura Stramonium*, to α-labelled tropic acid. He considers the possibility that it is formed from prephenic acid by condensation of the latter in the β-position with formaldehyde, or its biological equivalent, followed by decarboxylation and dehydration. This pathway appears unlikely because neither C^{14}-labelled formaldehyde nor C^{14}-labelled formate was incorporated into tropic acid and it would require the reversibility of the reaction prephenic acid → phenylalanine.

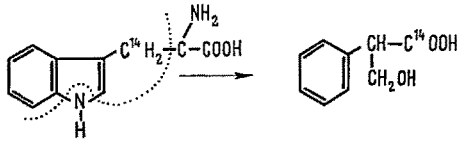
We have succeeded in showing that tryptophan acts as a direct precursor of tropic acid by loss of two terminal carbon atoms and elimination of the indole nitrogen.

On January 13, 1959, 50 μc of radiochemically pure DL-tryptophan-3-C^{14} was fed to 10 cm-tall plants of *Datura Stramonium* grown in aerated inorganic nutrient solution. Controls were run to assure complete absence of microbial conversion of the tryptophan. Absorption of tryptophan was ensured by replacing the absorbed solution with distilled water as needed. The plants were harvested on February 2, 1959, the alkaloid fraction separated and hyoscyamine isolated by the procedure of LEETE et al.[2]. The alkaloid was identified by means of its characteristic picrate salt and by co-chromatography with hyoscyamine (solvent systems: *n*-butanol, glacial acetic acid, water 4:1:1 (BAW) and water-saturated phenol).

Autoradiography showed the alkaloid to be radioactive. The hyoscyamine was eluted and hydrolyzed for 15 min with *N* 1 sodium hydroxide solution, the resulting tropine isolated by extraction with ether and then tropic acid with ether from the remaining acidified mixture. Both tropine and tropic acid were identified by means of chromatography (solvent systems: BAW and isopropanol, glacial acetic acid, water, 80:6:14). Autoradiograms showed that only tropic acid, not tropine was radioactive.

The radioactive tropic acid was mixed with 50 mg of tropic acid to act as a carrier, and the mixture was decarboxylated by heating with quinoline and powdered copper for 1 h at 230–235°C[7]. The evolved carbon dioxide was passed through a chilled trap, collected in *M* 1 Hyamine solution[8], and the radioactivity determined with a Packard Automatic Tri-Carb Liquid Scintillation Spectrometer. Residual traces of tropic acid in the styrene fraction were removed by shaking an ether solution of the styrene with dilute aqueous sodium hydroxide solution.

Thus, more than 99.3% of the radioactivity of the tropic acid resides in its carboxyl group, indicating the following biogenesis:



Zusammenfassung. Verfütterung von Tryptophan-3-C^{14} an *Datura Stramonium* führt zur Bildung von radioaktiver Tropinsäure, deren gesamte Radioaktivität auf die Carboxylgruppe beschränkt ist, der zu erwartenden Stelle bei direkter Umwandlung des Tryptophans unter Verlust des Stickstoffes und der zwei endständigen Kohlenstoffatome.

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Department of Biopharmacognosy, School of Pharmacy, Purdue University, Lafayette (Indiana), October 3, 1960.

[6] E. LEETE, J. Amer. chem. Soc. *82*, 612 (1960).
[7] C. WALLING and K. B. WOLFSTIRN, J. Amer. chem. Soc. *69*, 852 (1947).
[8] J. M. PASSMANN, N. S. RADIN, and J. A. D. COOPER, Anal. Chem. *28*, 484 (1956).
[9] This study was supported by grants from the Purdue Research Foundation and reported at the AAAS meeting, Chicago, December 1959.

Histochemical Observations on the Pigment Bodies of the Spinal Neurones of some Reptiles

The author in collaboration with GUPTA (GUPTA and SHARMA[1]) observed the presence of a pale-yellow, diffused pigment in the untreated living spinal neurones of the frog, *Rana tigrina*, studied with phase-contrast microscopy. This pigment is responsible for the general pale hue of the ganglia and is seen to occur in one or more areas in each cell. Quite often the pigment is observed in the interna of the duplex lipid bodies also.

The neurones of some reptiles, viz. ageing wall-lizards (*Hemidactylus flaviviridis* Rüppel), adult *Uromastix hardwickii*, and young and adult water-snakes (*Natrix piscator piscator* Schneider) studied by the author in the living condition under the phase-contrast microscope and with basic dyes used supervitally reveal discrete dirty-yellow to dark-brown pigment bodies and a refractile, pale-yellow substance—the pigment— confined to certain duplex lipid bodies only. The latter was, however, not seen in any of the fixed preparations. The living neurones of the water-snakes may reveal in addition a diffused pigment in the cytoplasm like that of frog. However, the general coloration of the ganglia in these reptiles is not pale-yellow in spite of the pigment in the neurones, unlike that in frog.

The pigment bodies in the reptilian neurones under discussion are distributed at random, although occasionally they tend to aggregate. These bodies exist as spheroids, subspheroids, and particulates with irregular contours measuring approximately 1 μ to 4 μ. Some of these bodies display variously distorted binary structures as well (Fig.). Curiously enough, the cells which abound in these pigment bodies rarely contain lipid particulates.

The pigment bodies resist all the fat solvents (e.g., cold and hot acetone, cold ethanol, chloroform, ether, and hot pyridine) and react negatively to most of the dyes and colorants. Only a few of them segregate neutral red (*super vitam*) and appear somewhat dirty-red. The various tests for carotenoids, such as Car-Price reaction[2], trichloroacetic acid[2], sulphuric acid[2], hydrochloric acid[2], and formic acid[2], when applied on the living ganglia, react negatively with these bodies. Similarly, LISON's test[3] for carotenoids tried on the gelatin sections of formaldehyde-calcium[4] fixed material also yields negative results.

[1] B. L. GUPTA and S. P. SHARMA, Res. Bull. Panj. Univ. *10*, 267 (1959).
[2] A. G. E. PEARSE, *Histochemistry* (Churchill Ltd., London 1960).
[3] L. LISON, *Histochimie et cytochimie animale* (Gauthier-Villars, Paris 1953).
[4] J. R. BAKER, Quart. J. micr. Sci. *90*, 293 (1949).