| | 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.

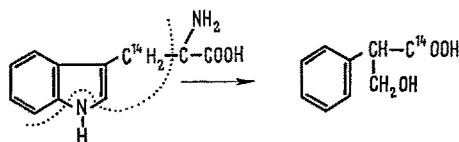
We have succeeded in showing that tryptophan acts as a direct precursor of tropic acid by loss of two terminal carbon atoms and elimination of the indole nitrogen.

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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## 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.

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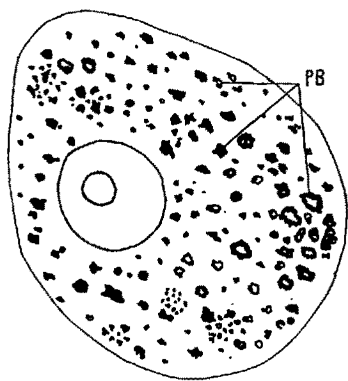


Diagrammatic representation of a reptilian spinal neurone showing the general distribution, and the various sizes and shapes of the pigment bodies (PB).

In formaldehyde-calcium/gelatin and Zenker-, Helly-, Bouin/paraffin sections of the ganglia coloured with ethanolic Sudan black B[4], some pigment bodies appear fairly coloured, while others even in the same site are only partly positive. These bodies give a faint red coloration in periodic acid-Schiff[5] and methyl green/pyronin G[6], react almost negatively to Sudan IV[2], and give a strong positive reaction with chrome alum haematoxylin[7]. The performic acid-Schiff[8] gives capricious results with these bodies, while the plasmal reaction[9,10] responds negatively.

The pigment bodies are, therefore, in all probability, some lipofuscins[2].

Besides these intracellular pigment bodies heaps of brownish-black, barrel- or spindle-shaped pigment bodies also occur surrounding the entire ganglion in the sections. It is noteworthy that unlike the above-mentioned intracellular pigment bodies these bodies defy all the histochemical reactions referred above.

In author's opinion the pigment bodies described above are not the products of secretion from the classical Golgi apparatus as claimed by MOUSSA and BANHAWY[11], since the author[12,13] has not come across any such apparatus or even a remotely comparable structure either in the living or in the processed young neurones. THOMAS[14,15], BAKER[16,17], NATH[18], and MALHOTRA[19,20], also hold identical views. The pigment bodies, on the other hand, appear to have originated as a result of partial or total oxidation of some of the lipid bodies. Lipid participation in the synthesis of pigment has also been advocated by COHN[21].

What, however, is the significance of this oxidation resulting in the accumulation of the pigment, in the economy of neurones, is a matter of speculation at this stage.

NAYAR[22] in the neurones of *Iphita limbata* Stal. (Hemiptera) considers the neurosecretory product as lipofuscins. Whether or not the above mentioned pigment bodies could be correlated with the neurosecretory product of Nayar in *I. limbata* Stal. is a matter of comprehensive investigation, but that the former bear a close histochemical relationship with the latter, inasmuch as both contain in them lipofuscins, is absolutely beyond doubt.

The refractile, pale-yellow pigment observed in the cores of certain duplex lipid bodies (*vide supra*) seems to have originated within the interna of these lipid particulates (Golgi bodies of HIRSCH) in much the same way as described by BAKER[4], HIRSCH[23], LACY[24,25], and KANWAR[26,27].

*Résumé.* Des lipofuscines, corps pigments jaune-sale à brun-sombre, ont été observées dans les neurones vivants et fixés de quelques reptiles. L'auteur y a aussi constaté la présence de corps pigmentés extracellulaires, noir-brunâtre, de nature histochimique inconnue.

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## The Lateral Transport of Indoleacetic Acid-$C^{14}$ in Geotropism[1]

The concept that the upward curvature of geotropically stimulated shoots results from the directed migration of auxin to the lower side has for over 30 years been a prominent feature of the theory of the tropisms. It was DOLK[2] who, by collecting auxin in agar blocks applied to the upper and lower parts of the basal surfaces of horizontal *Avena* coleoptile sections, and assaying the blocks by the standard *Avena* test, first provided clear-cut experimental support for this idea. He found that about 62% of the transported auxin was recovered from the block in contact with the lower side and 38% from that in contact with the upper side. This was true whether the source of auxin was the coleoptile tip, still attached, or, instead, agar containing exogenous auxin applied at the apical cut surface. Some recent workers, however, have suggested that DOLK's data need reinterpretation. They have applied $C^{14}$-labeled indoleacetic acid (IAA) to horizontally placed plant organs and, after bisecting these organs in the horizontal plane, have been unable to find any difference in radioactivity between the two sides. The results of BÜNNING et al.[3], CHING and FANG[4], REISENER[5], and especially of REISENER and SIMON[6] seem to show clearly that the total amount of auxin in the upper half of

[1] This work was supported by a National Science Foundation predoctoral fellowship to BARBARA GILLESPIE and by a grant from the National Science Foundation, No. G-9084, to Professor K. V. THIMANN.

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