An experimentally determined curve for p,p'-DDT is included in the figure for comparison. Points determined for p, p'-DDE and those obtained for the composite sample after dehydrohalogenation (4) are also included. These points fit the same curve and are shown thus in the figure.

It is noteworthy that the points obtained for the composite sample coincide with the p, p'-DDE curve in the range of 480-570 mµ. From 420 to 480 mµ some deviation occurs, but the points still closely approach the p,p'-DDE curve. Most of this deviation is due to the blank and becomes quite pronounced below 420 mµ. Dehydrohalogenation removes the blank effect in this range. Above 580 mµ appreciable divergence from the p,p'-DDE curve occurs, indicating the presence of p,p'-DDT. The blank effect has been found to be essentially negligible in this range of the spectrum. Two-component calculations, based on the absorption data of Fig. 1, showed 13.5  $\mu$ g of p,p'-DDT and 36.5  $\mu g p, p'$ -DDE in the aliquot analyzed.

This brief report strongly indicates that a large portion of the Schechter-Haller positive compounds occurring in human fat is a degradation product of DDT. The method of isolation used eliminates the two other major degradation products known to give essentially identical Schechter-Haller colors-namely, DDA and DBP. On this basis, it is tentatively believed the degradation product found is DDE. This observation, of course, does not eliminate the possibility that other degradation products may also occur in human fat. However, it seems unlikely that DDA would be deposited in adipose tissue in significant amounts, since it appears to be readily excreted under experimental conditions of DDT ingestion. As far as the writers are aware, DBP has yet to be demonstrated as an in vivo degradation product of DDT. Thus, the occurrence of significant quantities of this product in human fat would also seem doubtful.

The findings reported here raise several important questions. Presumably the DDT occurring in the fat of individuals of the general population arises mainly through contamination of a number of common foodstuffs. It is not known whether the DDE evidently present is also a contaminant as a result of partial degradation of the DDT residues on plant products prior to ingestion, or whether degradation occurs during digestion or after deposition in the fat. No direct experimental evidence appears available showing that ingested DDE is readily deposited in the fat. If DDT is slowly degraded after deposition in the fat, it would seem of great importance in assessing any potential danger from food contamination with DDT. In any case, the evidence for the occurrence of substantial proportions of DDE suggest that the possible health hazards involved in the widespread use of DDT need to be reconsidered and further investigated.

#### References

1. NEAL, P. A., et al. Public Health Repts., 61, 403 (1946). 2. STOHLMAN, E. F., and SMITH, M. I. J. Pharmacol. Exptl. Therap., 84, 375 (1945).

- LAUG, E. P., KUNZE, F. M., and PRICKETT, C. S., Arch. Ind. Hyg. Occupational Med., 3, 245 (1951).
  PRICKETT, C. S., KUNZE, F. M., and LAUG, E. P. J. Assoc. Offic. Agr. Chemists, 33, 880 (1950).
- 5. SCHECHTER, M. S., et al. Ind. Eng. Chem., Anal. Ed., 17, 704 (1945).
- 6. KNUDSON, H. W., MELOCHE, W. V., and JUDAY, C. Ibid. 12, 715 (1940).

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# The Microdetermination of "Free" L-Tryptophane in the Seedling of Lupinus albus

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Various authors (1-5) have reported the presence of indole-3-acetic acid or its derivatives in higher plants. In considering possible precursors of this auxin, we have investigated the distribution of Ltryptophane in the shoot of the seedling of Lupinus albus.

The method used in this work was to grow the seedlings in the greenhouse during the summer, to remove the various plant parts to be analyzed (apical meristems and leaf primordia were dissected under the binocular), to freeze them in a deep-freeze at  $-21^{\circ}$  C, and to lyophilize them to dry powders, which were then weighed in closed weighing bottles and stored in a desiccator.

The "free" tryptophane, i.e., the fraction which is not incorporated into proteins, was determined rather than the total tryptophane, because it was thought that this fraction was more readily available to give rise to the auxin. For this purpose, 0.3-25 mg of the dried material was placed in a centrifuge tube and boiled with about 6 ml of absolute ethanol until all the alcohol had evaporated. This was done in order to inactivate proteolytic enzymes which could liberate "bound" tryptophane in the course of the manipulations. About 3 ml of hot water was then added to extract the tryptophane, and the whole mixture was boiled for 3 min. The liquids and solids were then separated by centrifuging a few minutes at 1800 rpm. The supernatant was decanted, 2 ml of fresh boiling water was added to the residue, and the extraction procedure repeated once. The two liquid portions were brought together and their pH adjusted to 4.0 (glass electrode) with dilute HCl; then the solution was shaken with freshly redistilled ether in a separatory funnel to remove anthranilic acid which, along with indole, has been reported to interfere with the tryptophane assay (6). The pH of the aqueous fraction was then readjusted to 6.0 with NaOH, and the volume was completed to a given value with distilled water.

The concentration of L-tryptophane in this extract was estimated by a bacteriological method, using Lactobacillus arabinosus, ATCC #17-5. The pro-



FIG. 1. Proportionality between the amount of L-tryptophane added to the basic medium and the amount of 0.05 N NaOH used to bring the pH back to 6.0 after incubation with Lactobacillus (each point represents the mean of two titra-tions). The straight dotted line shows that the curve actually bends above the level of 1.0 µg of tryptophane.

cedure was somewhat refined over those previously described (7,8). The basal medium<sup>1</sup> used has a pH of 6.0 instead of 6.8. Into each test tube was pipetted 0.5, 1.0, or 1.5 ml of the plant extract, and the volume was completed to 3 ml with the correct concentration of basal medium. After autoclaving for 10 min, the tubes were inoculated and incubated 36 hr at 37° C. At the end of this period, the acidity was titrated with 0.05 NaOH to 6.0, using a Koch microburette, reading to 1/100 cc. At least 4 titrations (using 2 different concentrations of NaOH) were made on each unknown sample. Quantities as low as 0.1 µg of L-tryptophane can be quantitatively measured by this method, the proportionality between the amount of tryptophane and the volume of NaOH used remaining linear between 0 and about 1.0 µg Ltryptophane (Fig. 1) in 3 ml of solution (9).

The results obtained by this method with 14-day-old seedlings of L. albus are summarized in Table 1.

TABLE 1

"FREE" L-TRYPTOPHANE CONTAINED IN VARIOUS PARTS OF THE SHOOT OF 14-DAY-OLD L. albus SEEDLINGS

Plant part	Dry wt/ plant (in mg)	L-tryptophane	
		μg/100 mg dry wt	µg/plant
Apical meristem*	0.007	140.0	0.0098
Leaf primordia*	0.023	60.6	0.0139
Unfolded, hairy leaves	13.0	38.6	5.018
Large, expanded leaves	146.0	16.9	24.67
Stem (epicotvl)	7.64	69.6	5.317
Cotyledons	154.8	276.6	428.18

\* Indole and anthranilic acid not removed.

<sup>1</sup> Sold by H. M. Chemical Co., 1651 Eighteenth St., Santa Monica, Calif.

September 5, 1952

The concentration of a given compound in the tissues is often more important, physiologically speaking, than its total amount for the whole organ. The data of the third column which compare concentrations of tryptophane may, therefore, be more significant than those of the fourth column. The third column shows that the apical meristem-which is known to produce large amounts of auxin-is also well supplied with tryptophane. On the other hand, cotyledons seem to constitute an enormous reservoir of tryptophane, a fact of importance in the study of the tryptophane-auxin metabolism.

#### References

- 1. BERGER, J., and AVERY, G. S., JR. Am. J. Botany, 31, 199 (1944)2
- HAAGEN-SMIT, A. J., LEECH, W. D., and BERGREN, W. R.
- HAAGEN-SMIT, A. J., LEBER, W. D., and BERGER, W. R.
  Ibid., 29, 500 (1942).
  HAAGEN-SMIT, A. J., et al. Ibid., 33, 118 (1946).
  HOLLEY, R. W., et al. Arch. Biochem. Biophys., 32, 192 (1951)
- 5. REDEMANN, C. T., WITTWER, S. H., and SELL, H. M. Ibid., 80.
- 6. SNELL, E. E. Arch. Biochem., 2, 389 (1943).
- GREENE, R. D., and BLACK, A. J. Biol. Chem., 155, 1 7, (1944).
- WOOLEY, J. G., and SEBRELL, W. H. Ibid., 157, 141 (1945). 9. NITSCH, J. P. Compt. rend. soc. biol., 233, 1676 (1951).

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## Minimum Night Temperatures at or Near Full Moon

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Daily records of minimum temperatures have been kept during many years by observatories and meteorological stations, but such records have appeared in the form of long columns of numbers, among which an abnormality might remain unnoticed, unless special search were made for it. In a previous publication (1)from similar lists of minimum night temperatures taken in North Wales, monthly graphs were drawn, with temperatures as ordinates and days of the month as abscissae, for each of the years 1947, 1948, and 1949. When temperature curves were drawn, there appeared a regular fall in the minimum night temperature at or near the date of full moon at each lunation during the three years. This regularity was not noticed in the lists of numbers, but only in the monthly temperature curves. In order to conserve space, these were condensed to yearly graphs, which do not lend themselves to demonstration in so efficient a manner.

The temperature fall was sometimes gradual, its incidence being as much as a week before and its lowest point occurring on the date of full moon, or generally within 48 hr of it. Mostly, however, the curve of the fall was more sudden, being limited to 2 or 3 days. The lowest point of fall did not always coincide with full moon date, but happened within 2 or 3 days before, or occasionally after, it; so that, on that date, there was sometimes an incipient rise in the curve, or