droxymethylpyridine, 3-aminomethylpyridine dihydrochloride, 3-acetylpyridine and N-propyl-*o*-dihydronicotinamide. Included in the above list are several metabolites of nicotinamide and the interesting nicotinic acid antagonist, 3-acetylpyridine (*7*), but neither these compounds nor the others listed had any demonstrable effect in tuberculosis. Tuberculosis tests on several of the above materials have recently been reported by Fox (*8*).

The possibility that the antitubercular amides function by inhibition of nucleotidase was considered and rejected. Nicotinamide is a known inhibitor of this enzyme (*9–11*), but, although the inhibition by nicotinamide was duplicated (Table 1), it was found that pyrazinoic acid amide has no inhibitory action at similar levels. Furthermore, pyridazine-3-carboxamide is no more effective as an inhibitor than imidazole-4-carboxamide, which is not antitubercular.

The possibility that the active amides function by draining labile methyl was likewise disproved. Administration in diet of choline (0.2%), methionine (1%), and mixtures of these compounds (methionine 0.2%, choline 1%) had no effect on the antitubercular action of pyrazinoic acid amide. Also, it was found that high dosages of glycocyamine (1% in diet) did not influence tuberculosis infection in mice.

The pKa values of the various amides have been determined and are tabulated. There is, however, no obvious relation between these results and the tuberculosis data. More interesting, perhaps, are considerations of structure and reduction potential. In formation of pyridine nucleotides, nicotinamide is converted by reduction and alkylation to an N-phosphoribityl-3-carboxamido-1,2 (or 1,6)-dihydropyridinium moiety. Of the other heterocyclic amides only pyrazinoic acid amide and the 3- and 4-carboxamides of pyridazine are reducible to dihydro compounds (*12*). As the pyridazines undoubtedly reduce in the same way, judging by the similar reduction potentials, only one can have the same *o*-dihydro form as the reduced nicotinic and pyrazinoic acid amides. This suggests the possibility that the activity of the amides is due to excessive formation of pyridine or pseudopyridine nucleotide. The metabolism of the tuberculosis organism may be adversely affected either by this excess of nucleotide or by a resultant depletion of ribose or adenine required for other purposes. Related to this hypothesis, which is now being investigated, is the observation of McKenzie that riboflavin can replace approximately one third of the nicotinamide required in treatment of infected mice. We have not attempted to verify this but do not observe a similar supplementation with pyrazinoic acid amide.

The heterocyclic amides have been assayed microbiologically for nicotinic acid and antinicotinic acid activity. Although pyrazinoic acid has been reported effective in human pellagra (*13, 14*), neither pyrazinoic acid amide nor any other amide showed vitamin activity with the test organism, *Lactobacillus arabinosus*. Pyridazine-4-carboxamide was observed to inhibit slightly utilization of both nicotinic acid and nicotinamide, but the apparent antagonism may have been due to traces of pyridazine-4-carboxylic acid. This acid is a much more potent inhibitor. The inhibition index for both nicotinic acid and nicotinamide is approximately 7000 at half maximal inhibition.

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Examination of Human Fat for the Presence of DDT

G. W. Pearce, A. M. Mattson, and W. J. Hayes, Jr.

Communicable Disease Center, Public Health Service, Federal Security Agency, Savannah, Georgia

A program for analysis of DDT deposits in human fats was undertaken in this laboratory in 1949. The first samples examined were those from persons having known high exposure to DDT and other halogenated hydrocarbons. The material isolated from these fat samples showed the typical blue or blue-purple color given by DDT upon being analyzed by the Schechter-Haller method. However, upon extending this study to fat samples from persons having had no known excessive exposure to DDT or samples from persons who had not been exposed to large amounts of DDT for some time previous to biopsy, off-colors were encountered. The majority of these samples gave reds or purples of various intensities, indicating that the materials isolated contained a large proportion of degraded DDT.

It is noteworthy that in animals exposed experimentally to DDT it seems clearly established in the literature that DDT is stored primarily as such in the body fat. DDA [bis-(*p*-chlorophenyl) acetic acid] has been shown to be excreted in marked amounts in the urine of both man and animals under experimental conditions (*1, 2*) of DDT ingestion. In general, no evidence appears to have been presented indicating pronounced storage of degradation products of DDT in the body fat in the case of experimental animals.

The most extensive survey of the occurrence of DDT in fat samples taken from the general population in this country is that of Laug *et al.* (*3*). Unfortunately, these workers employed a method (*4*) that involved alkaline saponification of the fat with the simultaneous dehydrohalogenation of any DDT present. The dehydrohalogenated product was then isolated and subjected to the Schechter-Haller procedure (*5*) and measured as DDE [2,2-bis(*p*-chlorophenyl) 1,1-dichloroethylene]. Thus, any DDE present in the original fat could not be identified. The present paper is a preliminary report showing that in addition to DDT a degradation product, probably DDE, may occur in equal or greater amounts in human fat from the general population.

A modified Davidow H_2SO_4-celite column has been used to isolate the DDT and related products from CCl_4 extracts of the fat samples. Tests have shown that DDA and DBP (4,4′-dichlorobenzophenone) are retained by this column, whereas DDE and, of course, DDT appear quantitatively in the eluate. The CCl_4 has been removed from the eluates by distillation, and the residues have been subjected to a modified Schechter-Haller analysis (*5*). Spectrophotometric measurements have been made in 1-cm cells in a Beckman Model B at 520 mμ and 597 mμ. The colors developed have been treated as two color systems by the method of Knudson (*6*), the results being expressed as *p,p′*-DDT and *p,p′*-DDE. Because of obvious complications, no attempt has been made to measure or calculate the corresponding *o,p*-isomers potentially present in small proportions. The *p,p′* isomers of DDT and DDE have been used as standards, and good quantitative recoveries of them alone and in mixtures have been obtained when they were put through the entire procedure in the presence of both animal and human fat.

TABLE 1

DDT AND DDE ANALYSES OF HUMAN FAT SAMPLES

Sample No.	Micrograms			DDE (%)	DDT + DDE (ppm)
	DDT	DDE	DDT + DDE		
Group A					
1	1.9	3.3	5.2	64	3.6
2	10.4	13.0	23.4	56	54.6
3	3.4	1.7	5.1	33	6.3
4	8.4	7.2	15.6	46	12.8
Total	24.1	25.2	49.3		
Composite analysis	19.1	26.5	45.6		
Group B					
1	14.0	16.0	30.0	53	28.9
2	10.8	15.6	26.4	59	17.6
3	10.7	14.3	25.0	57	30.9
4	13.0	44.7	57.7	78	49.8
Total	48.5	90.6	139.1		
Composite analysis	42.8	92.2	135.0		

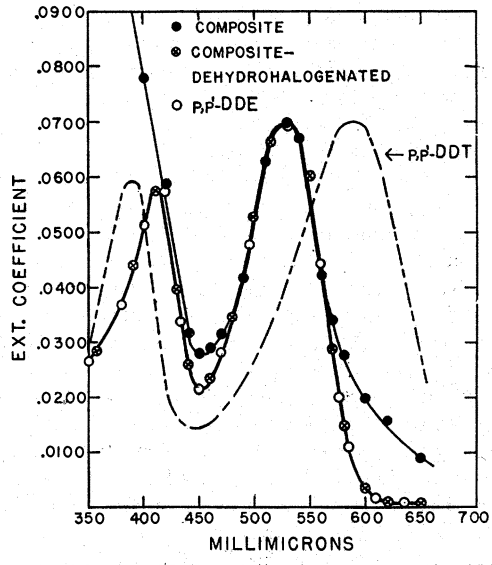

FIG. 1. Schechter-Haller absorption spectra of *p,p′*-DDT, *p,p′*-DDE, composite fat sample and composite sample after dehydrohalogenation.

Some typical analytical results on two groups of samples are presented in Table 1. After passing the carbon tetrachloride extract of each sample through the Davidow column, half of each eluate was analyzed, and the remaining halves were combined into two composite samples and also analyzed as indicated in the table.

The total micrograms of DDT and DDE arrived at by summation of the individual analyses agree very well with the corresponding composite analyses in the case of both groups. This indicates that the individual analyses are reasonably accurate. In comparing the sum of the individual analyses with their corresponding composite analysis, the DDE values agree more closely than the DDT results. It is believed that the composite analyses represent the more reliable data because of the higher concentrations.

The data in Table 1 show that among the eight samples the amount of DDE found ranges from 33 to 78% of the total, averaging 57%. Of approximately 60 samples of human fat examined in some detail, all have shown the presence of substantial amounts of DDE as indicated by the Schechter-Haller colors obtained. The proportion of DDE found has ranged from 33 to 90%. In general, most samples have been found to contain more DDE than DDT.

Absorption spectra of the Schechter-Haller colors obtained from a number of individual samples and a composite sample have been determined. In all instances these spectra have been found to approach the *p,p′*-DDE curve more closely than the *p,p′*-DDT. The data obtained on the composite sample illustrate this quite well and are presented in Fig. 1. The composite sample was obtained from 16 fat samples having 2–20 ppm of DDT and DDE. The combining technique was the same as that used in the case of the composite samples shown in Table 1.

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 μg of *p,p'*-DDT and 36.5 μg *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.

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

J. P. Nitsch and R. H. Wetmore

Biological Laboratories,
Harvard University, Cambridge, Massachusetts

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 L-tryptophane 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° 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-