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Preliminary observations of the effect of DDT ingestion on the soluble proteins of rat liver

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IT HAS been observed that the ingestion of DDT results in a marked decrease in liver glucose-6-phosphate dehydrogenase,[1] an enzyme found in the soluble fraction. This observation has prompted a study of the distribution of soluble liver proteins on modified cellulose columns. Studies have been made with preparations from animals raised on control rations or rations containing 200 ppm DDT.

Male rats weighing 100 g were raised for 4 weeks on a semisynthetic ration[1] or a ration containing 200 ppm DDT. At the end of this period the animals were sacrificed, the livers excised, and 10% homogenates prepared in 0·25 M sucrose buffered to pH 7·4 with 0·005 M phosphate. Mitochondria, nuclei, and cell debris were removed by centrifuging at 10,000 *g* for 10 min. Centrifuging at 100,000 *g* for 30 min sedimented the microsomal fraction, and the soluble fraction remaining was used for these studies.

The soluble fraction was then treated with solid ammonium sulfate and the protein fraction recovered between 40% and 80% saturation was used for cellulose chromatography. One could expect a large proportion of the enzyme protein to be recovered in this fraction. The protein was redissolved in 0·005 M phosphate buffer at pH 7·0. The residual ammonium sulfate was removed by applying to a Sephadex G-25 column and eluting with buffer.

After this preliminary treatment the protein solution was applied to a DEAE-cellulose column (2·0 × 40 cm), prepared as described by Seal & Gutman.[2] The protein was eluted with 0·005 M phosphate buffer, pH 7·0 with a linear sodium chloride gradient. The effluent concentration of sodium chloride increased from 0·015 M at tube 35 to 0·46 M at tube 260. In the different phases of these experiments protein concentration was monitored by observing the absorbance at 280 mμ. Protein recoveries from both the Sephadex and cellulose columns ranged from 95–98%.

This level of DDT in the ration for the feeding period does not cause a change in growth rate but does result in a 35% increase in liver size. Also, the proportions of protein recovered at the different levels of ammonium sulfate saturation were not modified to any extent by DDT ingestion; approximately 32–38% of the soluble protein was recovered in the fraction from 40–80% saturation. The data illustrated in Fig. 1 show that DDT ingestion markedly affects the distribution of the particular protein fraction when chromatographed on DEAE cellulose. With the preparation from the control rat a major portion of the protein is eluted in tubes 40–60. Preparations from DDT-fed rats do not give this fraction, but a series of smaller peaks is eluted at higher sodium chloride concentrations. These experiments have been repeated with three rats from each dietary group, and distribution comparable to those given in Fig. 1 have been obtained. From the 260/280-mμ ratio it appears that the last fraction to be eluted is nucleoprotein.

The observed change of protein distribution could result from the direct interaction of DDT or its metabolites with the soluble proteins, or it is possible that different proteins are synthesized under this stress. In the latter case DDT might interact in some fashion with the reactions of protein synthesis. If DDT or its metabolites were adsorbed on a protein surface, a change in tertiary structure could produce a changed charge distribution and consequent changes in elution pattern on a cellulose

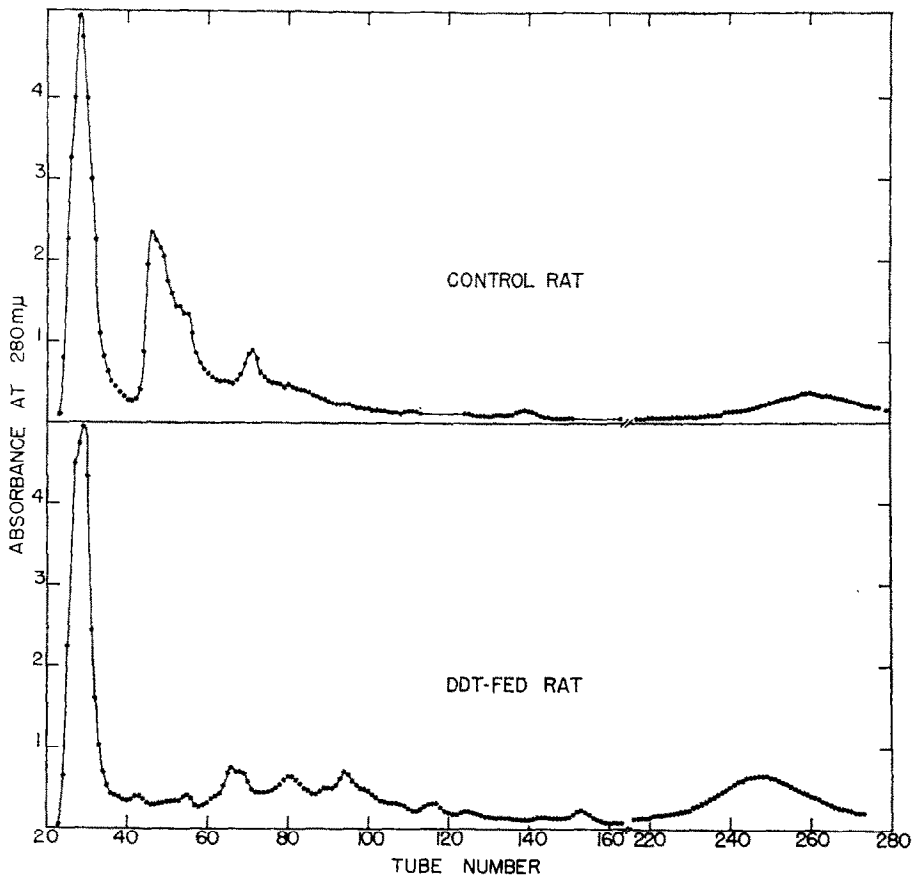

FIG. 1. Chromatography of soluble proteins of liver on DEAE-cellulose. Effect of DDT ingestion on protein distribution.

column. It is also possible that adsorption of DDT might produce a dissociation of multi-chain proteins, as has been reported for certain organic solvents[3] and hormones.[4]

These studies are of a preliminary nature, and numerous other variables could be evaluated. The technique does show promise of being a valuable approach to describing metabolic changes *in vivo* caused by toxic chemicals.

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