ties of the mucin fraction.

Summary. 1. Human respiratory tract mucin possesses a resistance lowering factor similar in activity to that of hog gastric mucin. This effect was shown by intrabronchial inoculation of rats with pneumococci suspended in saline or the two types of mucin. It was also shown by intraperitoneal inoculation of mice with streptococci suspended in saline, in two types of mucin and in fractions thereof. 2. Evidence against resistance lowering properties of mucin depending on the presence of blood group A antigen was obtained by fractionating human respiratory tract mucin and testing the fractions for blood group A antigen and the resistance lowering properties of the fractions.

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Tissue Distribution, Accumulation and Elimination of the Isomers of Benzene Hexachloride. (18631)

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One of the properties of benzene hexachloride which makes it an effective agricultural insecticide is the relatively persistent action of residues of this material remaining after spraying or dusting. However, this property may give rise to a consumer hazard through the ingestion of foodstuffs contaminated by these residues. In order to evaluate the potential consumer hazard acute(1) and chronic (2) toxicity experiments have been conducted. It was noted that in single oral doses the toxicity of the isomers decreased in the order of gamma, alpha, delta and beta. However, in the chronic experiments it was observed that the beta isomer was the most toxic followed by alpha, gamma and delta. An explanation of this phenomenon was sought by investigating the tissue distribution, the relative degree of accumulation after feeding diets containing the various isomers, and the rate of elimination of the isomers from the fat after removal of the benzene hexachloride from the diet. Early work from this laboratory on the distribution of the gamma isomer indicated that it might be stored preferentially in the fat(3).

Method. Rats used in this study were from our stock of Osborne-Mendel strain and they were fed *ad libitum* a diet consisting of ground commercial laboratory chow to which had been added the various isomers of benzene hexachloride dissolved in a small quantity of corn oil. The analytical method employed was that of Davidow and Woodard which depends upon the conversion of the benzene hexachloride to 1,2,4-trichlorobenzene and the determination of the latter by its characteristic ultraviolet spectrum(4). To determine the tissue distribution of benzene hexachloride, weanling rats were placed on diets containing 800 p.p.m. of the alpha, gamma and delta isomers, and 100 p.p.m. of the beta isomer. Feeding of these diets was continued for approximately 20 months after which time the rats were sacrificed and the tissues removed. Dogs weighing from 11 to 16 kg were given gelatin capsules of corn oil solutions containing 5% of the alpha isomer, 2% of the gamma isomer, or 10% of the delta isomer. Due to

* Portions of this paper are excerpts from a thesis submitted by John P. Frawley in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Georgetown University.

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the relative insolubility of the beta isomer in corn oil it was incorporated into ground commercial laboratory diet at a level of 0.2%. The dosages of the various isomers fed the dogs were selected so as to obtain an approximately equivalent degree of severe toxicity. For the accumulation study, rats ranging in weight from 140 to 200 g were fed diets containing 100 p.p.m. of the individual isomers of benzene hexachloride. At two-week intervals 2 male and 2 female rats were sacrificed for each of the diets. The abdominal fat from each rat was removed and analyzed for benzene hexachloride. For the elimination study, weanling rats ranging in weight from 50 to 80 g were placed on a diet containing 100 p.p.m. of the individual isomers of benzene hexachloride for a period of 6 weeks in order to obtain maximum storage. At the end of this time the diets were replaced by control diets. Two female and 2 male rats on each of the diets were sacrificed at the end of the 6-week storage period, and a similar number at weekly intervals thereafter. The abdominal fat was removed from the animals and analyzed for benzene hexachloride.

Results. If benzene hexachloride had been stored in the tissue as the 1,2,4-trichlorobenzene a chromatographed hexane solution of the tissues would have exhibited the characteristic absorption curve of the 1,2,4-trichlorobenzene(5). An examination of the absorption spectra showed that this was not the case. However, when the material in the hexane solution was refluxed with alkali it then exhibited the characteristic absorption curve of 1,2,4-trichlorobenzene, thus indicating that these isomers of benzene hexachloride were stored unchanged in the adipose tissue.

Tissue distribution. Analyses of tissues from 2 female rats on each diet (Table I) indicated that the greatest storage was to be found in the fat tissue; brain, kidney, liver and muscle showed the presence of some benzene hexachloride, but considerably less. The tissue distribution data for dogs are summarized in Table II. The dosages for the dogs fed the beta isomer were calculated from food

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TABLE I. Tissue Distribution of Isomers of Benzene Hexachloride in Female Rats After 20 Months' Feeding.*

	800 p.p.m. diet level			100 p.p.m. diet level
	Alpha (p.p.m.)	Gamma (p.p.m.)	Delta (p.p.m.)	Beta (p.p.m.)
Fat	3500	440	550	1900
Brain	230	73	75	130
Kidney	180	75	97	150
Liver	120	Neg.	20	20
Muscle	75	20	38	75

* Each figure represents average storage for 2 animals.

consumption and average weight data. This data shows that the isomers of benzene hexachloride are preferentially stored in the adipose tissue of dogs. The alpha and beta isomers are stored to a lesser degree in liver, kidneys and adrenals, while the gamma isomer is stored to some extent in the kidneys and the adrenals. The liver, kidneys and adrenals of dogs fed the delta isomer show no detectable storage.

Accumulation. The results of adipose tissue analyses on rats receiving benzene hexachloride in their diet and the striking differences in storage among the isomers can be seen in Fig. 1. It can also be seen that the concentrations of the isomers in the fat of female rats are generally higher than in the fat of males. It will be noted that the storage concentration of the isomers, with the possible exception of beta isomer, tends to level off between the fourth and sixth week and that no significant increase in accumulation appears after this point.

Elimination. The results of the analyses conducted on the abdominal fat of rats fed benzene hexachloride in their diet are best illustrated by Fig. 2. This figure clearly shows the rapid elimination of the alpha, gamma and delta isomers, and the persistent nature of the beta. In the case of the female rats fed the beta isomer, small amounts to the extent of 40 p.p.m. are still present after 14 weeks' feeding of a control diet.

Discussion. Although the number of dogs used in the distribution study is small, it would appear that there are certain differences between rats and dogs in degree of storage in

TABLE II. Tissue Distribution of the Isomers of Benzene Hexachloride in Dogs.

Isomer	No. of dog	Dose, mg/kg	Weeks on exp.	Tissue distribution,* p.p.m. Fat	Liver	Kidney	Adrenals
Alpha	1	60	12	3870	1520	215	2600
Beta	2	24, 30	19, 20	8825	1100	50	505
Gamma	4	10, 15	2–7	466	Neg.	140	372
Delta	2	120	14, 20	550	Neg.	Neg.	Neg.

* Each figure represents average storage.

the various organs. For example, in the case of the alpha and beta isomers, the relation between storage in livers and kidneys is reversed for the 2 species. Another species difference was observed in the sensitivity of dogs to the gamma isomer, as is noted in Table II. However, this comparison is complicated by the fact that the dogs received more toxic doses of the isomers than the rats and died (2-20 weeks) before having had opportunity

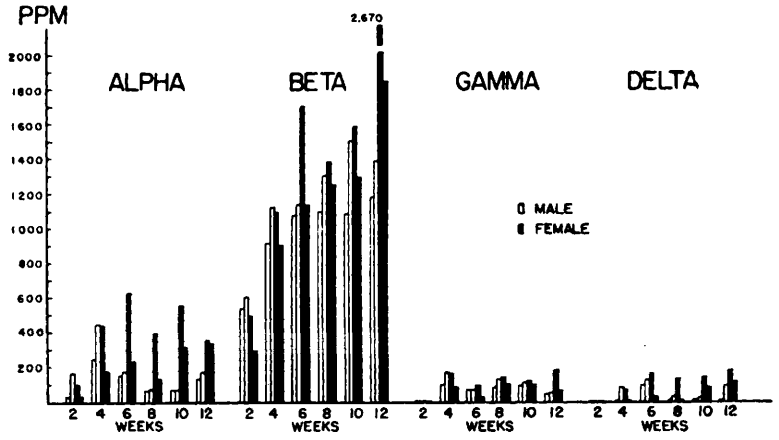

FIG. 1.

Accumulation of the isomers of benzene hexachloride in fat tissue of rats fed 100 p.p.m.

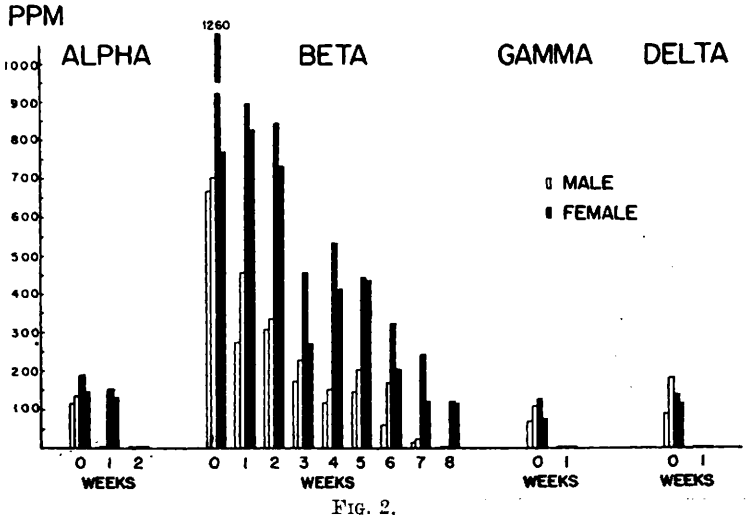

FIG. 2.

Disappearance of the isomers of benzene hexachloride from fat tissue of rats following the removal of the toxicant from the diet.

to develop a characteristic picture of long-term effects. Certainly, other factors than mere storage in the fat determine the toxicity of compounds ingested over long periods of time. However, we believe that here we have an example of a parallelism between gross signs of chronic toxicity and tissue accumulation.

Summary. 1. The alpha, beta, gamma and delta isomers of benzene hexachloride are preferentially stored in the adipose tissue of rats and dogs. 2. Some storage of benzene hexachloride is found in kidneys, brain, liver and muscle tissue of rats. 3. Dogs show considerable storage of the alpha, beta and gamma isomers in the adrenals. 4. The degree of accumulation in the adipose tissue bears a direct relationship to the chronic toxicity of the isomers. 5. The relative rates of accumulation in rat adipose tissue show that the approximate maximum storage is accomplished within 6 weeks. 6. Upon removal of the benzene hexachloride diet, alpha, gamma and delta isomers disappear from rat fat depots within 3 weeks; the beta still persists in small amounts after 14 weeks.

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Amino Acid Composition of Dentin Protein.* (18632)

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The nature of the protein in the enamel of human teeth has been studied by Losee and Hess(1), Losee, Neidig, and Hess(2), and Block, Horwitt, and Bolling(3). From these investigations it appears that enamel protein is an eukeratin according to the criteria of Block. Block *et al.*(3) have also investigated the nature of human dentin protein by the technic of paper chromatography applied to the hydrolysate of dentin protein prepared by us and, although their results were qualitative, they have classified it as a collagen. Using quantitative colorimetric, chromatographic, and microbiological methods we have determined a number of the amino acids present in dentin protein.

Experimental. The protein was prepared from dentin ground from the fully erupted, permanent, non-carious first and second human molars used for the isolation of enamel protein(1). The teeth were cut at the cementoenamel junction and only the crown was employed. The ground dentin was dried in a desiccator, over anhydrous calcium chloride, for 48 hours and then demineralized by the method used to prepare enamel protein(1). The isolated protein was dried in the same manner as the original dentin. The yield of dentin protein obtained in several separate experiments was between 10 and 11 percent based upon the dry weight of the dentin. The sample of dentin protein used for the various determinations of the amino acids contained 2.06% ash and 16.09% nitrogen calculated upon the weight of the desiccator dried sample. Three methods of analysis were employed: colorimetric procedures applied directly to an acid hydrolysate of the protein, colorimetric procedures upon eluates resulting from chromatographic separation of the amino acids from the hydrolysate

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