was measured on an Aminco microphotometer (American Instrument Company, Inc.), the signal of which was fed into a Health servo recorder (model EUW 20H).

No fluorescence was detected when either the albumin alone or the ANS alone was adsorbed to either type of BLM. However, after flushing the membrane with 0.1*M* KCl or 0.1*M* acetate buffer solution and subsequently adding the other one of the pair of compounds (Fig. 2, point A), we observed enhanced fluorescence. The intensity of the fluorescence response (Fig. 2) depends on which of the two compounds is adsorbed onto the phospholipid membrane first. For example, when ANS is adsorbed onto the membrane first, and then bovine serum albumin (BSA), is added (curve I), the fluorescence response is three times larger than in the case (curve II) where BSA is adsorbed onto the BLM before ANS is added. If the phospholipid BLM is again flushed with 0.1*M* KCl solution (point B), the fluorescence decreases to half its original intensity in the first case, whereas, after a second flushing with KCl solution in the second case (curve II), the fluorescence returns almost to the base line. The maximum increase in fluorescence for the situation depicted in curve I occurs when the *p*H of the surrounding solution is between 5.6 and 7.

When the BLM is prepared from either of the solutions of oxidized cholesterol saturated with ANS, subsequent addition of BSA does not cause a significant change in the fluorescence. Moreover, BLM formed from oxidized cholesterol alone shows no increased fluorescence, regardless of the order of addition of ANS and BSA.

Three important conclusions can be drawn from these results. First, ANS must adsorb to the phospholipid portion of the BLM, but not to the cholesterol or oxidized cholesterol portion, or both. However, the orientation of the ANS bound to the lecithin must be such that the emitting moiety projects into the aqueous phase, since there is no enhancement of fluorescence over that observed in water alone.

Second, either the enhanced fluorescence observed upon the addition of H_2O has been excluded from the ANS or the protein surrounds the emitting moiety with hydrophobic groups upon adsorption. The latter explanation seems most likely since the enhancement of fluorescence is greatest in the *p*H range 5.6 to 7 (if the *p*H at the interface is about 2 *p*H units lower than that of the solution, then this corresponds to the maximum *p*H range for BSA-ANS interaction in solution). Finally, the lack of enhanced fluorescence when ANS is added to the BLM after the addition of BSA indicates that the protein adsorbs to the BLM in such a way that the hydrophobic regions are not available for ANS penetration.

Changes in fluorescence dependent on *p*H can be related to the conformational changes in serum albumin itself. Enhancement of fluorescence was observed around *p*H 3.35 (*7*) because of an increase in the number of apolar sites in albumin. However, thus far we have not been able to perform experiments on the enhancement of fluorescence at low *p*H because of the instability of BLM at low *p*H.

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24 November 1969

Chlorinated Hydrocarbon Insecticides: Root Uptake versus Vapor Contamination of Soybean Foliage

Abstract. *The major environmental source of DDT (dichlorodiphenyltrichloroethane) residues in soybean plants arises from vapor movement from contaminated soil surfaces. In contrast, the presence of dieldrin, endrin, and heptachlor results primarily from root uptake and translocation through stems to leaves and seeds.*

The chlorinated hydrocarbon insecticides have been among the most useful pesticides for the protection of agricultural crops during the last two decades. Nevertheless, advances in instrumentation during the last 6 to 8 years have demonstrated that minute quantities of these insecticides may contaminate our foods and feeds.

Crops may become contaminated

Table 1. Soybean sorption of [^{14}C]DDT, [^{14}C]dieldrin, [^{14}C]endrin, and [^{14}C]heptachlor residues from soils and percentage of residue as insecticide. Values for the insecticides have been adjusted so that direct comparison among insecticide residues is possible. All values are the mean of four replications, except those for dieldrin and heptachlor sorbed through roots which are based on three replications. Values in parentheses represent the percentage of total residues as parent insecticide.

Plant part	Insecticide [counts per minute (in thousands) per gram of dry weight]			
	DDT	Dieldrin	Endrin	Heptachlor
		Through roots		
Upper leaves	0.8	1.1	34.1	26.6
Lower leaves	1.4 (0.3)	6.2 (64.6)	62.4 (19.0)	36.6 (0.1)
Upper stem	0.6	8.2	84.5	14.7
Lower stem	0.8 (Trace)	61.7 (91.1)	139.8 (57.3)	33.9 (4.5)
Pods	0.1	0.3	4.1	3.9
Seeds	0.1 (None)	0.2 (117.2)	1.3 (2.7)	1.8 (0.7)
		Through vaporization		
Upper leaves	4.5	10.2	6.2	4.7
Lower leaves	9.2 (77.9)	16.6 (51.9)	13.1 (51.6)	9.8 (2.3)
Upper stem	0.8	0.9	0.9	1.0
Lower stem	0.7 (46.2)	0.6 (107.2)	0.6 (41.3)	1.1 (21.3)
Pods	1.1	1.2	1.1	1.2
Seeds	0.1 (None)	0.3 (91.4)	0.4 (31.0)	0.6 (5.1)

with the chlorinated insecticides in several ways. (i) The insecticide may be applied directly to the crop, be sorbed, and then persist in the plant. (ii) The plant may be contaminated by insecticide drift or by dust particles that contain adsorbed insecticide. (iii) Roots may remove insecticides from soils and translocate them within the plant. (iv) The plant foliage may sorb insecticides vaporized from the soil, although this has not been demonstrated.

Our experiment, which demonstrates that plants can become contaminated with chlorinated hydrocarbon insecticides vaporized from soil, was designed to permit the investigator to distinguish between the contribution of root uptake and sorption of vapors by aerial plant parts. In this paper the term "residue" refers to the parent insecticide and to its degradation products.

Soybeans were grown in nonsterile soils subjected to either a surface or a subsurface treatment (Fig. 1) of 20 parts per million (about 45 kg/ha) of DDT, dieldrin, endrin, or heptachlor (*1*). The purity of the applied insecticide was 99+ percent. In surface-treated soil systems no portion of the plant came in contact with the surface soil. The stem was protected from the soil by growing through a glass tube. Splashing was avoided during irrigation. In subsurface-treated soil systems the soybean roots were in intimate contact with the treated soil. Surface and subsurface soils were separated by a fiberboard and paraffin seal. Insecticide residues vaporized from the surface soil treatment were localized around the plant by a wire-supported plastic cage.

The treated surface layer of 250 g of Lakeland sandy loam soil contained either 0.5 μc of [^{14}C]DDT, [^{14}C]dieldrin, or [^{14}C]endrin, or 0.2 μc of [^{14}C]heptachlor per 100 g of soil. The same concentrations of ^{14}C-insecticide were used in the subsurface layer of 1500 g of soil. Treated layers contained sufficient nonlabeled insecticide to give 20 ppm. The insecticides were thoroughly mixed into the soil.

The soybean plants were harvested after 53 days and separated into upper leaves, upper stem, lower leaves, lower stem, pods, and seeds. A portion of each plant part was combusted and the evolved CO_2 containing ^{14}C was trapped; the radioactivity was determined by liquid scintillation techniques. The leaves and stems from each plant were bulked. Leaves and stems were extracted with a mixture of hexane, acetone, and methanol (8:1:1, by volume), and the extracts were partially purified and analyzed by gas-liquid chromatography (*2*).

Table 1 gives the radioactive content in counts per minute in the various plant parts. These counts represent those of both the parent insecticides and their degraded products. Root uptake and translocation accounted for the greatest amounts of dieldrin, endrin, and heptachlor residues found in soybean tops. Sorption of DDT residues vaporized from surface-treated soil was 6.8 times greater than that obtained through root uptake and translocation of subsurface treatment. Most of the dieldrin and endrin residues sorbed through the roots were found in the lower stem. Heptachlor and DDT residues were more evenly distributed throughout the stems and leaves when sorbed through the roots.

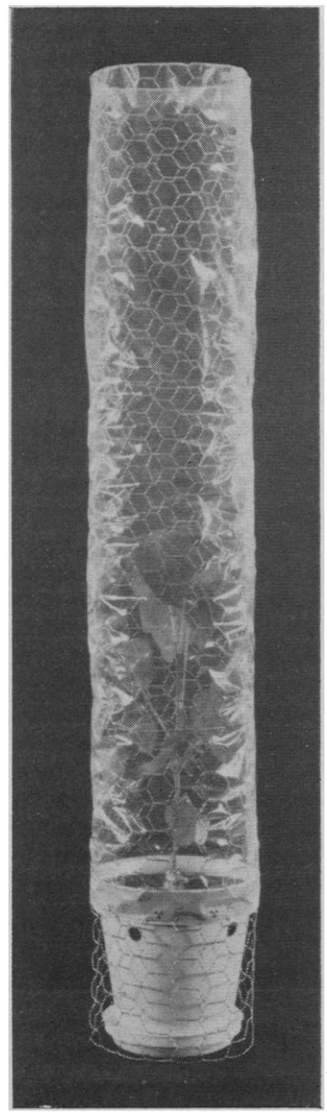

Fig. 1. Potted soybean plant used in an experiment to distinguish between root sorption of insecticides and sorption of insecticide vapors contaminating aerial plant parts. Surface and subsurface soils were separated by a sealed disk with no part of the plant contacting the surface-treated soil.

The lower leaves contained most of the residues, and the upper leaves contained the next largest amount of the residues, from the surface treatments; DDT residues in stems were comparable for both surface and subsurface treatments, whereas root uptake and translocation accounted for the major portion of dieldrin, endrin, and heptachlor residues in stem tissue. Presumably the stems, which have considerably less surface area than the leaves, did not significantly sorb these insecticides. Unfortunately, the plastic cages were discarded and not assayed for ^{14}C-residue adsorption.

Although the vapor pressures of these insecticides (in millimeters of mercury at 25°C) are different (DDT, 2×10^{-7}; dieldrin, 7.8×10^{-7}; endrin, 2×10^{-7}; heptachlor, 4×10^{-4}) (*3*), the residues found in the various plant parts from the surface treatment were surprisingly near the same order of magnitude. It is unclear why DDT and endrin, with the lowest vapor pressures, should cause the same amount of foliar contamination as heptachlor. Several explanations seem possible. Heptachlor may have been degraded more rapidly to a lower vapor pressure product; it may have desorbed from the leaves as rapidly as it was adsorbed; or maybe a major portion of the heptachlor was vaporized and escaped before the soybean plant was large enough to intercept the heptachlor residues. We kept the surface moist by watering it once or twice a day, and the moisture may have influenced vaporization of these chemicals (*4*).

Table 1 also contains the percentage of total residues as parent insecticide. These values were obtained by gas-liquid chromatographic assay of the partially purified soybean extracts. Only dieldrin residues in stems and seeds were fully recovered as dieldrin. The DDT constituted 78 and 46 percent of the total DDT residues in the leaves and stems, respectively, in plants contaminated through vaporization, whereas detection of any DDT from plants contaminated through root uptake was questionable.

These results demonstrate that plants can be contaminated with DDT,

dieldrin, endrin, and heptachlor residues by vaporization from soil surfaces as well as from root uptake and translocation. The major source of DDT contamination is vaporization from the soil. Although earlier researchers have shown that dieldrin concentration in leaves was 50 times higher from field-grown corn than from greenhouse-grown corn protected from aerial contamination (*5*), they did not demonstrate that this large difference was the result of dieldrin vaporizing from the soil.

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12 December 1969; revised 28 January 1970 ■

Insulin Release from Isolated Human Fetal Pancreatic Islets

Abstract. *Pancreases were obtained from five human fetuses 12 to 16 weeks old. The islets of Langerhans were isolated with collagenase, and then incubated with buffer, glucose, tolbutamide, or glucagon added to the medium. The insulin released into the medium was measured by immunoassay. Glucagon produced the only significant increase above base line; glucose and tolbutamide failed to enhance secretion of insulin. The data suggest that isolated human fetal islets of this gestational age develop responsiveness to glucagon earlier than to glucose or tolbutamide.*

In the human fetus the islets of Langerhans can be detected by the 12th week of pregnancy; pancreatic insulin can be demonstrated either by acid-ethanol extraction or with fluorescent antibody (*1*, *2*). Circulating insulin has been measured as early as 12 weeks (*2*, *3*). To our knowledge, the responsiveness of the isolated human fetal pancreatic islets to various stimuli has not been studied, although the newborn has been investigated intensively. We now report studies on isolated human fetal islets challenged with glucose, tolbutamide, or glucagon.

Therapeutic abortion was performed on five nondiabetic women; gestational age was estimated by fetal measurement and ranged from 12 to 16 weeks. The fetal pancreas was placed in cold Hanks solution (*p*H 7.4) and transported to the laboratory. The interval between completion of the abortion and initiation of incubation with collagenase was less than 20 minutes. Islets were isolated as described by Lacy and Kostianovsky (*4*) with the following modifications: 15 mg of collagenase (5) per pancreas was used, and the incubation time necessary to obtain adequate digestion was extended to 40 minutes. The islets could easily be recognized under the dissecting microscope. They were cleaned and removed from exocrine tissue with two needles and transferred with a 100 μl pipette into small tubes containing Krebs-Ringer bicarbonate (KRB) buffer with 2 percent bovine serum albumin. The *p*H was adjusted to 7.4 with 1*N* NaOH. Additions to the KRB buffer were as follows: glucose (3 mg/ml), sodium tolbutamide (0.1 mg/ml), and glucagon (0.005 mg/ml).

Each pancreas supplied a minimum of 16 islets. Because of the very limited number, we placed only two islets in each incubation flask; however, all incubations were performed in duplicate and are reported as the mean of these duplicate determinations. Each experiment contained four groups: buffer alone; buffer and glucose; buffer and tolbutamide; and buffer and glucagon. The final volume was 1 ml per flask. All flasks were incubated at 37°C in a Dubnoff metabolic shaker for 90 minutes under continuous gassing with 95 percent O_2 and 5 percent CO_2. Afterward the islets were allowed to settle for 20 minutes at room temperature. Two 0.1-ml aliquots were then removed for insulin determination in duplicate by a double antibody immunoassay (*6*) with human insulin used as a standard. To assess any possible contamination of glucagon with insulin, we also incubated control tubes, without islets, from each experimental group. In addition, a recovery study was performed by adding human insulin to glucagon which was of bovine-porcine origin.

The recovery of human insulin added to glucagon was adequate (data not shown). Immunological analysis of the control glucagon samples revealed insulin contamination; 5 μg of glucagon contained between 5 to 15 μU of immunoreactive insulin (IRI) per milliliter; therefore, 15 μU of IRI were subtracted when glucagon was present in the incubation medium. The mean basal insulin secretion was 41 ± 9 μU/ml (Table 1). The addition of glucose (3 mg/ml) induced no significant change from basal insulin secretion, although three out of five exhibited slightly higher values. The mean insulin secretion was 45 ± 8 μU/ml. Tolbutamide also failed

Table 1. Insulin release by isolated human fetal pancreatic islets. Each value represents the mean of two experiments. Within parentheses are individual values. Results are expressed as microunits of immunoreactive insulin (IRI) per milliliter released into the incubation medium. The values for glucagon have been corrected for possible insulin contamination. The mean and standard error of the mean for each group are: buffer, 41 ± 9 μU/ml; glucose, 45 ± 8 μU/ml; tolbutamide 35 ± 9 μU/ml; glucagon, 70 ± 12 μU/ml.

Fetus No.	Fetal age (weeks)	Buffer	IRI released (μU/ml)		
			Glucose (3 mg/ml)	Tolbutamide (0.1 mg/ml)	Glucagon (0.005 mg/ml)
1	12	30 (45, 15)	30 (30, 30)	40 (45, 35)	55 (50, 60)
2	14	50 (40, 60)	60 (50, 70)	20 (15, 25)	75 (75, 75)
3	15	35 (25, 45)	45 (40, 50)	20 (15, 25)	60 (55, 65)
4	16	70 (65, 75)	60 (60, 60)	70 (65, 75)	115 (100, 130)
5	16	20 (20, 20)	30 (20, 40)	25 (20, 30)	45 (40, 50)