prepared in petroleum ether and absolute alcohol was placed in a solution containing only buffer at *p*H 7.0 for the same time. These sections were then again placed in absolute alcohol overnight and afterward stained with the periodic acid-Schiff reaction. Another section was placed in absolute alcohol overnight and stained with PAS to show the total PAS-reactive material. Sections were counter-stained with malachite green. Sections from the original Kirkman tumor and from twenty transplants performed in our laboratory were examined.

Dark purplish-red granules, which are black in the accompanying photomicrographs, may be seen in the large cells which are scattered sparsely throughout the tissue section (Fig. 1). Clusters of these cells are present not only in the connective tissue septa between tumor cell masses (see arrow, Fig. 1), but also in the tumor mass (Fig. 2). The PAS-positive granules in these cells were removed by the diastase digestion but were not removed by the buffer alone. Most of the tumor cells in this study were not PAS-positive although Kirkman (*2*) found the majority of hamster renal tumor cells to be PAS-positive. The glycogen cells described here were found in the autonomous and the estrogen-dependent tumors and were seen in all the sections examined.

These cytochemical studies thus show that glycogen is present in some of the cells of estrogen-induced renal tumors in the hamster. Others have not found glycogen in these tumors, but the methods of fixation that they used may have removed this relatively soluble carbohydrate from the tissue section. Fortner (*5*) described clusters of large polygonal cells in a bile-induced (*6*) renal tumor in the hamster. These cells contained large amounts of clear or granular cytoplasm and could, indeed, represent the glycogen-containing cells described in this paper. The question naturally arises as to whether these glycogen-containing cells are altered tumor cells, or specialized cells from the connective tissue matrix. The preponderance of these cells in the connective tissue septa suggests that they may arise from this connective tissue matrix.

Renal tumors in hamsters and in humans are morphologically similar. Since they are histochemically similar as evidenced by the presence of glycogen-containing cells in both, the question arises as to whether the mechanisms of tumor induction are also similar. Thus, mechanisms of tumorigenesis may be elucidated by a study of the intermediate pathways of glycogen metabolism of these tumors (*7*).

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Chlorinated Insecticides in the Body Fat of People in the United States

Abstract. *Benzene hexachloride and dieldrin are present in the body fat of people in the general population of the United States. The mean concentration of dieldrin is 0.15 ± 0.02 parts per million, which is in good agreement with the concentration reported for southern England. The mean concentration of benzene hexachloride is 0.20 ± 0.04 ppm, which is considerably lower than the mean concentration reported for France. Paired analyses of DDT by gas chromatographic and colorimetric methods show that the results of the latter may give incorrectly high results when applied to human fat.*

Both DDT [1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl) ethane] and its metabolite DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethylene] are present in the body fat of people in the general population of the United States (*1, 2*) and other countries (*3–5*). It was shown recently, by the use of gas chromatography and highly sensitive detectors, that dieldrin (*4*) (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene) and benzene hexachloride (*5*) (1,2,3,4,5,6-hexachlorocyclohexane), respectively, are present in the body fat of people in southern England and France. No information has been available as to the occurrence of chlorinated pesticides, other than DDT and DDE, in the body fat of the general population of this country, but, because the average concentrations reported for DDT and DDE in the United States (*2*) were higher than those reported for other countries (*3–5*), we suspected that other chlorinated pesticides might also be present. Therefore, in connection with a periodic survey carried out in 1961–62 to determine whether there has been any change in the storage of DDT in people of this country, those samples of fat that were large enough were analyzed not only by the Schechter-Haller method (*6*) but also by microcoulometric gas chromatography (*7*) to detect other insecticides.

The results obtained by the Schechter-Haller method (*6*) for DDT and DDE in 131 samples from the general population and 51 other samples from people with occupational or other special exposure are to be reported separately (*8*). The two autopsy and 28 surgical samples reported here (Table 1) were collected in Wenatchee, Washington; Phoenix, Arizona; and Louisville, Kentucky. All but four of the subjects from whom the samples were obtained had been residents of the respective states at least 2 years; only two (samples 29 and 30) had experienced occupational exposure to pesticides. In no instance was illness related to pesticides.

For this study, samples of body fat (18 to 42 g) were taken for comparative study by Schechter-Haller colorimetric analysis, and by gas and paper chromatography. These samples were chopped and extracted in a Waring blender with *n*-hexane. After extraction was complete, sodium sulfate was added and mixing was continued for 5 minutes to remove moisture. The extracts were then filtered and given a preliminary cleaning by partitioning with acetonitrile (*9*).

Gas chromatograms (Fig. 1, *A* and *D*) of standard solutions showed that dieldrin and DDE have the same retention times on the columns used. Since we were interested in determining both the dieldrin and DDE in fat by gas chromatography, a preliminary separation of these compounds was required.

A column in which florisil is used has been described by McKinley *et al.*

Table 1. Concentrations of chlorinated hydrocarbon pesticides in the body fat of people in the United States, estimated by microcoulometric gas chromatography and by Schechter-Haller colorimetric analysis, and expressed as parts per million. (General population except where noted.)

Sample No.	Lipid (%)	Microcoulometric gas chromatography						Colorimetric analysis		
		BHC	Dieldrin	*p,p'*-DDE	*o,p'*-DDT	*p,p'*-DDT	Total as DDT	DDE	DDT	Total as DDT
		Samples collected in Wenatchee, Washington								
1	88	0.15	0.14	2.35	0.63	0.50	3.74	3.7	2.5	6.6
2	68	0.15	0.27	2.00	<*el**	0.86	3.09	4.0	1.9	6.3
3	75	0.11	0.16	10.00	5.00	1.25	17.39	17.8	8.1	27.9
4	66	<*el*	0.25	1.72	<*el*	0.73	2.65	4.0	2.0	6.4
5	58	0.70	<*el*	3.38	<*el*	1.04	4.80	6.7	2.9	10.4
6	60	0.50	0.36	3.80	<*el*	1.18	5.41	4.9	1.9	7.3
7	46	<*el*	0.23	1.86	0.92	0.73	3.72	3.8	2.3	6.5
8	62	<*el*	0.23	5.14	2.20	1.63	9.55	3.8	2.7	6.9
9	50	<*el*	0.20	2.76	<*el*	1.66	4.73	6.9	4.1	11.8
10	70	0.27	0.32	10.40	2.43	3.25	17.27	8.6	4.8	14.4
11		<*el*	<*el*	0.76	<*el*	<*el*	0.85	2.1	0.7	3.0
		Samples collected in Phoenix, Arizona								
12	70	0.67	0.27	6.52	3.44	2.93	13.63	6.9	6.4	14.1
13	75	<*el*	0.34	9.07	2.27	4.97	17.34	9.7	9.8	20.6
14	74	<*el*	0.18	1.83	1.51	0.68	4.23	5.5	2.5	8.6
15	74	0.69	0.18	3.38	1.51	1.16	6.43	2.8	1.1	4.2
16	52	0.20	0.09	3.17	0.88	1.16	5.57	4.5	2.3	7.3
		Samples collected in Louisville, Kentucky								
17	76	0.33	0.07	8.94	3.40	3.06	16.42	22.4	10.7	35.6
18	42	<*el*	<*el*	0.69	<*el*	<*el*	0.77	4.1	0.4	5.0
19	63	0.14	0.11	1.34	<*el*	0.48	1.97	2.1	0.9	3.2
20	60	0.22	0.14	3.04	1.12	1.13	5.64	4.4	3.2	8.1
21	86	0.24	0.09	1.73	<*el*	0.47	2.40	5.2	3.9	9.7
22		0.19	0.16	1.94	<*el*	0.70	2.86	5.8	3.3	9.8
23		0.41	0.04	6.00	<*el*	1.79	8.47	11.7	9.1	22.1
24	42	<*el*	<*el*	6.76	8.24	<*el*	15.77	12.5	11.0	24.9
25	65	<*el*	<*el*	5.73	0.95	0.98	8.31	4.5	3.6	8.6
26	58	0.09	0.02	0.48	0.35	0.23	1.11	1.5	0.9	2.6
27	67	<*el*	<*el*	0.55	<*el*	<*el*	0.61	2.1	1.2	3.5
28		0.20	0.11	2.10	0.44	0.52	3.30	2.8	1.3	4.4
		Samples from people who had experienced occupational exposure to pesticides								
29†	72	0.36	0.27	11.80†	2.71†	2.54†	18.39†	15.2†	12.1†	29.0†
30‡	72	0.47	0.36‡	3.28	2.43	<*el*	6.08	6.0	2.0	10.8
		Mean ± standard error§								
		0.2	0.15	3.82	1.30	1.14	6.69	6.23	3.71	10.71
		±0.04	±0.02	±0.54	±0.35	±0.21	±1.02	±0.88	±0.55	±1.51

* Experimental limits (*el*) by gas chromatography at 128 ohm, 5 mv: BHC, 0.03 ppm; dieldrin, 0.02 ppm; *p,p'*-DDE, 0.06 ppm; *o,p'*-DDT, 0.09 ppm; *p,p'*-DDT, 0.09 ppm. † Applicator of DDT more than one year before sample taken. ‡ Applicator of aldrin within last year. § Means and standard errors exclude values for people who experienced occupational exposure.

(*10*) for the separation of DDT, DDE, and DDD [1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethane] from dieldrin, methoxychlor [1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl) ethane], and Kelthane [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethanol]. A similar column was used in the present work. Florisil, 60/100 mesh, was used, reactivated at 210°C for 3 hours in a vacuum oven. A column with an inner diameter of 19 mm, and measuring 250 mm in length, was packed dry with 14 cm of florisil. About 1.5 cm of anhydrous sodium sulfate was then added and the column was packed by gentle tapping of the side walls. Glass wool was used to retain the column pack. Enough benzene wetted the column to ensure uptake. Samples dissolved in benzene were added to the column. It was found that benzene hexachloride (BHC), DDT, DDE, DDD, aldrin, heptachlor, and heptachlor epoxide could be eluted quantitatively (95 to 100 percent) in the first 100 ml of benzene. Each column was further eluted with 50 ml more of benzene, and this eluate was found to contain no pesticide. This gave assurance of complete separation of these materials from dieldrin and methoxychlor, which were finally eluted quantitatively (99 to 100 percent) from the column with 100 ml of 1 percent acetonitrile in acetone.

The benzene eluates of fat samples from this column were suitable for analysis. However, the acetonitrile-acetone eluates containing dieldrin required further cleaning. This was accomplished by an alkaline saponification followed by elution of the saponification products that were soluble in *n*-hexane, through an alumina-packed column with ether-hexane as described by Cueto (*11*).

Samples of each of the clean benzene eluates and the ether-hexane eluates were then separately subjected to microcoulometric gas chromatography (*7*); the samples of the benzene eluates were also analyzed by the Schechter-Haller method. To confirm the identity of materials present in fat, qualitative results were obtained from two different columns and under different operating conditions (Fig. 1). Benzene hexachloride (peak 1) was present in 19 of 30 samples; *p,p'*-DDE (peak 3 in chromatograms *B* and *E*) was present in all samples. Dieldrin (peak 3 in chromatograms *C* and *F*) was found in 25 of the 30 samples. Peak 4, which principally represents *o,p'*-DDT as no *p,p'*-DDD was found by paper chromatography (*12*), appeared in 18 of the 30 samples; *p,p'*-DDT (peak 5) was present in 26 of 30 samples. Quantitative results obtained by gas chromatography are presented in Table 1.

The mean concentration of dieldrin (0.15 ± 0.02 ppm) reported in this paper is in good agreement with that reported (*4*) for southern England. The mean concentration of BHC (0.20 ± 0.04 ppm) is considerably lower than the 1.19 ppm reported (*5*) for the French. The mean concentrations of *p,p'*-DDE, *o,p'*-DDT and *p,p'*-DDT in individuals who had not knowingly been exposed to DDT were 3.82 ppm, 1.30 ppm, and 1.14 ppm, respectively. There was no significant difference in the storage of BHC ($p = > .1$) or DDT ($p = > .5$) based on geographic source; however, the samples from Louisville, Kentucky, and nearby places contained significantly less dieldrin ($p = < .005$) than those from Phoenix, Arizona. The man with recent occupational exposure to dieldrin stored no more than one other subject who had not, to his knowledge, been specially exposed to it. The man who experienced occupational exposure to DDT more than a year earlier showed storage near the top of the range of the general population.

It was found that the ratio of *o,p'* to *p,p'* isomers of DDT stored in fat is not the same as their ratio in technical grade DDT. Since the Schechter-Haller method (with or without modification) was used in previous analyses of human fat for DDT-derived materials, there was no way of measuring the storage ratio of the isomers, but this ratio influences quantitative results obtained by the method. By necessity, the assumption that the *o,p'* and *p,p'* isomers of DDT would be stored in the fat in the proportion they occur in technical DDT (20 : 80) (*1*) was used in developing a two-color equation for the determination of *p,p'*-DDE and technical DDT (*6*). Among fat samples from 30 people, we found more of the *o,p'*-DDT isomer in 10 of the 16 indi-

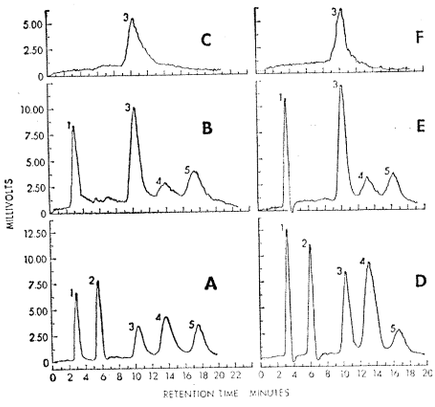

Fig. 1. Gas-coulometric chromatograms of a standard solution (*A* and *D*), and portions of a single extract of human fat (*B*, *C* and *E*, *F*) passed through two columns. First column (*A* to *C*): 1.22 m by 0.635 cm outer diameter, packed with 5 percent Dow oil grease on 30/60 mesh acid-washed Chromasorb P, at a column temperature of 182°C, nitrogen being used as a carrier gas at a flow rate of 88 cm³/min. Second column (*D* to *F*): aluminum, 1.83 m by 0.635 cm outer diameter, packed with 20 percent high-vacuum silicone grease on 30/60 mesh acid-washed Chromasorb P, at a column temperature of 215°C, nitrogen being used as a carrier gas at a flow rate of 240 cm³/min. The standard solution produced five peaks in chromatograms *A* and *D* representing: 1, benzene hexachloride; 2, aldrin; 3, dieldrin plus *p,p'*-DDE; 4, *o,p'*-DDT plus *p,p'*-DDD; and 5, *p,p'*-DDT. Chromatograms *B* and *E* were obtained from samples of the first 100 ml of benzene eluate from a florisil column, while chromatograms *C* and *F* were from samples of the final (acetone-acetonitrile) eluate from the same column. Peak No. 3 represents DDE in chromatograms *B* and *E* and dieldrin in chromatograms *C* and *F*.

viduals showing both isomers in their fat and in two others who showed no *p,p'* isomer, with an average of 1.54 times as much *o,p'*-DDT as *p,p'*DDT in the 18 people and 1.31 times as much in the entire group.

Standards were run by Schechter-Haller analysis in which the ratios of *o,p'*- to *p,p'*-DDT were varied while holding DDE constant and then applying the two-color equation for technical DDT and *p,p'*-DDE. When the ratios of *o,p'*- to *p,p'*-DDT were 20 : 80, 50 : 50, and 80 : 20, respectively, the recoveries of DDE and DDT were 97 and 109 percent, 118 and 94 percent, and 131 and 72 percent, respectively. These recoveries were obtained when the amount of DDE was aproximately equal to the sum of the *o,p'*- and *p,p'*-DDT isomers. When DDE was decreased to one-half the sum of the *o,p'*- and *p,p'*-DDT isomers, and the isomer ratio was 80 : 20, the recoveries of DDE and DDT were 188 percent and 71 percent, respectively. This means that for fat samples in which *o,p'*-DDT is increased relative to *p,p'*-DDT (in comparison to technical grade DDT used in establishing the two-color equation), results obtained by the Schechter-Haller method reflect higher DDE and lower DDT than is actually present.

The average total DDT-derived material expressed as DDT (6.69 ± 1.02 ppm) found by gas chromatography was only 62 percent of that found by the Schechter-Haller method for the same eluates (Table 1) and the difference is significant ($p = < .05$). Since recovery of standards by gas chromatography was good (95 to 100 percent), the differences obtained by the two methods when the same eluates were used must have been due to one or both of the following reasons: (i) Schechter-Haller calculation with equations based on a 20 : 80 storage ratio of *o,p'*- to *p,p'*-DDT, and (ii) variations in Schechter-Haller blanks.

By gas chromatography, DDE calculated as DDT averaged 62 percent of the total DDT-derived materials and agrees well with the 58 percent average reported for DDE by Hayes *et al.* (*2*).

Aldrin, heptachlor, heptachlor epoxide, and methoxychlor were not detected in any of the samples, but would have been detected had they been present in concentrations similar to those of BHC, dieldrin, and DDT-derived compounds.

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Partial Synchronization of Nuclear Divisions in Root Meristems with 5-Aminouracil

Abstract. *Root tip cells of* Vicia faba *were partially synchronized in nuclear stages by treatment for 24 hours with 700 parts of 5-aminouracil per million. All division was suppressed by the analog treatment, and a peak in division stages (up to 62.5 percent) was reached 14 hours after removal from the aminouracil. Populations of partially synchronized cells can be useful in experiments designed to study various intracellular reactions or responses at different stages in the nuclear cycle.*

Partial synchrony of nuclear divisions has been achieved in root tip meristems of *Vicia faba* (broad bean) by treatment with 5-amino-2,4-dioxypyrimidine or 5-aminouracil (5AU).

Two types of experiments were performed. The first was designed to determine 5AU concentrations that would synchronize mitoses by stopping cell divisions and then, when treatment was terminated, would result in synchronous resumption of mitoses giving an eventual maximum proportion of dividing nuclear stages. The purpose of the second set of experiments, in which autoradiography with tritiated thymidine was used, was to study DNA synthesis after treatment with 5AU.

Roots of *Vicia faba* were grown in half-strength Hoagland's nutrient solution, treated with 5AU, washed in water, and then transferred back to nutrient solution for further growth. All treatment and recovery solutions were aerated and maintained at 20°C in the dark. All 5AU treatments were of 24 hours' duration, and the solutions contained 700 ppm of the pyrimidine base in deionized water. Exploratory experiments indicated that the specific time and concentration were not critical, but those used appear to be effective maxima. For the mitotic synchronization studies, summarized in Fig. 1, secondary roots were fixed in modified Carnoy's solution (3:2:1), stained with Feulgen, and squashed on slides.

For autoradiographic studies (Table