or H_2O. The zinc may be removed by adding a small amount (1 to 100 ml) of 0.1*M* sodium citrate to the zinc-virus compound followed by dialysis against H_2O or 0.85-percent NaCl. An alternate method is the removal of zinc utilizing ion-exchange resins (*3*). Analyses show that either dialysis or resin exchange will remove zinc to a residual concentration of less than $2 \times 10^{-5}M$.

Titration of the virus concentrate in monkey kidney tissue culture demonstrates that no loss of virus occurs. The titers expressed in infective units for dose $\log_{10}$ of type II MEF_1 virus before zinc precipitation, and of the supernatant after zinc precipitation were $10^{7.7}$ and $10^{4.2}$, respectively, and of type III Saukett were $10^{5.5}$ and $10^{1.5}$, respectively, or approximately 99.9 percent of the virus was removed. The stability of the zinc precipitate has been followed for 10 wk at 4°C without perceptible loss of infectivity for monkey kidney tissue culture. (It is interesting to note that the titer of the virus is unaltered even when zinc is combined with it.)

The method described here has many obvious applications. It provides a technique for safety testing of the finished vaccine, since any desired concentration may be effected. The process provides a simple way of removing various components of the medium, such as antibiotics, amino acids, and metabolites. It should simplify the production of the vaccine, since large volumes of virus may be conveniently and rapidly concentrated for processing and killing. This should make possible better standardization of virus inactivation with formaldehyde on other agents. With the removal of the growth medium, selection of a preservative and antigenic stability of the vaccine may no longer be a problem.

The method would appear to have wide application. Highly purified, stable herpes simplex virus has been obtained (*4*).

A complete report concerning work with poliomyelitis viruses is in preparation.

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Some Analogs and Derivatives of the Insecticide DDT

A casual inspection of recent chemical literature reveals that no fewer than 102 organic compounds, either isomeric or analogous DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane], have been prepared. An inclusion of related condensation products would provide even a far more imposing list. We have recently prepared five additional analogs of DDT that have not been previously reported (*1*). In connection with the preparation of these five compounds, a more exhaustive study has been made of several other variations.

The general method of preparation consisted in the condensation of chloral hydrate, bromal, or butyl chloral with several mono- or disubstituted benzenes in the presence of fuming sulfuric acid. In several instances, the resulting compounds were subsequently treated with alcoholic potassium hydroxide in order to eliminate hydrogen halide. These ethylenic products were then further halogenated or oxidized to related derivatives. Various nitro derivatives were also prepared by the direct nitration of the original condensation products. The results of 33 types of attempted condensations under a variety of conditions that involved several hundred syntheses seemed to indicate that chloral hydrate and monohalogenated benzenes condense readily in the presence of fuming sulfuric acid. Bromal and monohalogenated aromatic compounds formed condensation products that were, in general, relatively unstable, as was previously reported (*2*). Butyl chloral and chlorobenzene formed condensation products of unknown and variable structure. Ethyl benzoate and chloral hydrate formed an apparent condensation product that was unstable to treatment with alkali. The condensation of chloral hydrate with *p*-dichlorobenzene, *p*-dibromobenzene, and *p*-chlorobromobenzene formed products, the structure of which has not been fully established. *m*-Dichlorobenzene, *o*-dichlorobenzene, and *o*-chlorotoluene did not condense readily with chloral or its hydrate under comparable conditions using fuming sulfuric acid.

The specific experimental data in the preparation of these analogs is as follows.

1) 1,1-Dichloro-2,2-bis(*p*-iodophenyl)-ethylene: A mixture of 10 g of 1,1,1-trichloro-2,2-bis(*p*-iodophenyl)ethane (*3*) and 6 g of potassium hydroxide in 400 ml of 95-percent ethanol was refluxed for 4 hr. The reaction mixture was poured into ice water, filtered, and recrystallized from absolute ethanol, yielding 4.8 g (51-percent theory) of product; melting point, 146.4 to 147.0°C (corrected). As proof of structure, this compound was oxidized with chromium trioxide in glacial acetic acid to *p-p'*-diiodo benzophenone; melting point, 235° to 236°C (*4*).

2) 1,1,1-Trichloro-2,2-bis(3-nitro-4-iodophenyl)ethane: A solution of 10 g 1,1,1-trichloro-2,2-bis(*p*-iodophenyl)-ethane in 160 ml of glacial acetic acid and 160 ml of fuming nitric acid (specific gravity, 1.60) was heated on a steam bath for 5 hr. The resulting nitrated product was poured into ice water, filtered, and recrystallized from absolute ethanol, yielding 5 g (45-percent theory) of product; melting point, 173.7° to 174.3°C (corrected). It was assumed that one nitro group entered the 3-position *o*- to the iodine. Analytic calculated for $C_{14}H_7Cl_3I_2N_2O_4$: N, 4.46 percent; found: 4.52 percent.

3) 1,1,1,2-Tetrachloro-2,2-bis(*p*-bromophenyl)ethane: A warm solution of 20 g of 1,1,1-trichloro-2,2-bis(*p*-bromophenyl)ethane (*5*) in carbon tetrachloride was chlorinated with strong illumination during 4 hr. The carbon tetrachloride was removed by vacuum distillation, and the solid was recrystallized from absolute ethanol, yielding 12 g (57-percent theory) of product; melting point, 95° to 96°C. Theoretical silver halide for $C_{14}H_8Cl_4Br_2$: 1.980 g/g of compound; found: 2.018 g/g of compound.

4) 1,1,1-tribromo-2,2-bis[2(5)-chloro-5(2)-bromophenyl]ethane: A 1.0- to 2.0-mole ratio of bromal and *p*-chlorobromobenzene was heated at 85° to 90°C while 400 ml of 15-percent fuming sulfuric acid was slowly added with vigorous stirring. The reaction mixture was heated for an additional 2 hr at the same temperature, resulting in the formation of a yellow precipitate. The product was filtered and then washed, first with 10-percent sodium bicarbonate solution and then with water. After recrystallization from a 50–50 mixture of ethanol and trichloroethylene, followed by absolute ethanol, 50 g (50-percent theory) of a yellow product was obtained, melting point, 163.0° to 163.7°C (corrected). Theoretical silver halide for $C_{14}H_7Cl_2Br_5$: 1.898 g/g of compound; found: 1.895 g/g of compound. We are inclined, in the light of orientating tendencies and steric hindrance, to assume that the coupling in the phenyl group is *o*- to the chlorine and *m*- to the bromine, although the converse might be true. Final confirmation on this structure is still lacking.

5) 1,1,1-Tribromo-2,2-bis(2,5-dibromophenyl)ethane: A similar condensation was made with a 1.0- to 2.0-mole ratio of bromal and *p*-dibromobenzene that was heated with 720 ml of 15-percent fuming sulfuric acid and stirred at 75° to 80°C for 2 hr. The brown prod-

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uct was filtered and then washed, first with 10-percent sodium bicarbonate solution and then with water. After crystallization from a 50–50 mixture of ethanol and trichloroethylene, followed by recrystallization from ethanol, a 140-g yield (19-percent theory) was obtained; melting point, 179.8° to 180.6°C (corrected). Analytic calculated for $C_{14}H_7Br_7$: Br, 76.15 percent; found: Br 76.87 percent.

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Intracellular Symbiosis in Cockroaches: II. Mitotic Division of Mycetocytes

In part I of this series, we describe the histology, slow growth, and poor reproductive performance of aposymbiotic German cockroach nymphs (*Blattella germanica* L.) (*1*). These were produced by continuously feeding their parents Aureomycin, an antibiotic that prevents the ovarial transmission of the symbiotes (bacteroids). In connection with that work, stained serial sections of a few normal control roaches showed various stages of mitosis among the mycetocytes (the symbiote-containing cells of the fat body, Fig. 1.) Figures 4 and 5 are photomicrographs of representative dividing mycetocytes. Such cells arrested our attention (*2*), because the failure of earlier authors to find mitosis had suggested that the mycetocytes increase by amitosis during nymphal growth (*3*).

The division of the mycetocytes was synchronized so that numerous mitotic figures were distributed throughout the entire abdomen of all nymphs showing mitoses. In most nymphs, no divisions were seen. Bursts of mitotic activity are known in other organisms, so we made a series of preparations of nymphs of known ages to see whether the bursts were correlated with the molting cycle.

Under our rearing conditions, nymphs molt approximately every 10 or 11 days, passing through six molts and maturing at about 60 to 65 days. A total of 80 nymphs, ranging from newly hatched to 40 days, most of them in the second instar, were sectioned and stained. All of the sections of the abdomens were inspected. Counts were made of nymphs with the most easily recognizable stages —that is, metaphase and anaphase. A total of 14 nymphs showed mitotic mycetocytes on the 9th, 16th, 18th, 20th, 30th, and 40th days. In the second instar, of 14 nymphs between the ages of 11 to 14 days, inclusively, there were no mitoses; while of 24 nymphs between the ages of 16 to 20 days, inclusively, there were 10 with mitoses.

It was clear that mitosis occurs in bursts in the latter half of the instar. Frequently mitosis in the mycetocytes was accompanied by mitosis in the mid-gut epithelium and in other cells of the fat body, either urate- or fat-cells (indistinguishable in young nymphs). Divisions were not noticed in the epidermis. Undoubtedly mitosis has escaped previous detection because it occurs in nymphs of an age not usually studied.

Mitotic processes were also seen in mycetocytes and other fat-body cells in nymphs of the wood roach, *Parcoblatta pennsylvanica* (DeGeer), which had been collected while they were hibernating in February and kept active in the laboratory until they were sacrificed in June.

Mycetocytes differentiate in aposymbiotic embryos—that is, in embryos developed free of bacteroids. These "empty mycetocytes" are present as clusters of cells with large nuclei and small amounts of fibrous-appearing cytoplasm devoid of bacteroids (Fig. 2). There are 10 clusters, one in each lateral half of abdominal segments 2 through 6. The positions of the empty cells are the same as the positions of the mycetocytes, which in normal embryos are formed in anticipation of their infection by the bacteroids (*3*). The empty mycetocytes were never seen dividing. Seemingly, without the pressure of a growing population of bacteroids, the mycetocytes lack the stimulus to divide and separate; thus they remain in clusters and at their original number.

An interesting contrast to the empty mycetocytes was the behavior of certain bacteroid-containing mycetocytes that grew to gigantic proportions in other experimental roaches. Serial sections of various developmental stages following short Aureomycin treatment (*1*) showed that some individuals were deficient only in bacteroids; that is, they were semi-aposymbiotic. Only a small fraction of the normal complement of bacteroids had been transmitted to the nymphs; these bacteroids were present as subnormal populations in the cytoplasm of just a few mycetocytes. The few mycetocytes eventually grew, by the end of 9 wk, to about 200 times the normal volume instead of dividing (Fig. 3). Perhaps this phenomenon may be accounted for by a postulated lack of a dividing stimulus, correlated with an inadequate bacteroid population when the cells were young and ordinarily would have undergone division. There seemed to be no check on the multiplication of the bacteroids.

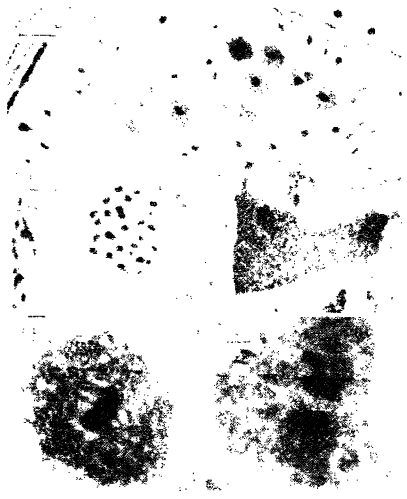

Fig. 1. Part of a cross section of a 2-wk old normal nymph showing distribution and size of mycetocytes. Fig. 2. Part of a cross section of a 2-wk old aposymbiotic nymph showing one cluster of empty mycetocytes. Fig. 3. Section through a giant mycetocyte in a 9-wk old retarded nymph. Figs. 4 and 5. Photomicrographs of sections through mycetocytes whose nuclei are undergoing mitosis. The bacteroids are so numerous that the spindle fibers are almost obscured. The bacteroids look hollow as a result of the fixing and straining procedure. All material was fixed in Carnoy's fluid, sectioned at 10 μ, and stained with Delafield's hematoxylin, counterstained with erythrosin in clove oil.

We conclude that mitosis is the normal means of division in mycetocytes and that it occurs in bursts near the end of each instar. If only a few bacteroids are present originally, the bacteroids increase but the mycetocytes do not divide, whereas if bacteroids are absent the mycetocytes remain small, in clusters, and do not divide.

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