Table 1. Hemagglutination by rabbit serum after immunization with antigens from stages of the life-cycle of *S. mansoni* and *S. douthitti*.

Antigen	No. injected	Hemagglutination titer
Male worms, *S. douthitti**	230, 585	1:20, 1:80
Female worms, *S. douthitti**	234	1:40
Male and female worms, *S. douthitti**	218	1:160
Male and female worms, *S. douthitti*†	3 ml of 1:100 by weight	1:40
Female worms, *S. mansoni**	345	1:160
Immature female worms, *S. mansoni**	115	0
Male worms, *S. mansoni**	530	1:160
Cercariae, *S. mansoni**	12,000, 180,000	1:20, 1:80
Cercariae, *S. mansoni*†	6 mg in saline	1:20, 1:40
Cercariae, *S. mansoni*‡	18,000, 21,000	1:80, 1:160
Miracidia, *S. douthitti*§	1900	0
Spleens of mice infected with many eggs of *S. douthitti*†	14.4 ml 1:100 by weight	1:640

* Saline homogenate. † Lyophylized antigen.
‡ Frozen cercariae in aquarium water. § Living miracidia in aquarium water.

tosomatium douthitti (Table 1), and for the serums of six hosts immunized twice weekly for 3 wk with various doses of frozen cercariae of *S. mansoni* (Table 2). Control serums of all the afore-mentioned hosts were negative by hemagglutination. All serums in Table 1 were tested a minimum of four times, and the titers recorded are averages. Since the number of rabbits immunized with a particular antigen is small, the titer for each animal immunized is listed separately.

One rabbit immunized with frozen cercariae of *S. mansoni* showed a titer of 1:80. This animal was reimmunized 3 mo later, and the titer rose to 1:640. Agglutinins were not detected in rabbits immunized with living miracidia of *S. douthitti* and with immature female worms of *S. mansoni*. Agglutinins were not detected in the serum of a rabbit immunized with an antigen prepared from a frog lung trematode (*Haematoloechus* sp.). The titer of 1:640 obtained after immunization with a lyophylized mouse spleen-egg antigen was unexpected, since this serum showed no *in vitro* activity against living cercariae and very little activity in immobilizing miracidia (*8*).

Although saline extracts of frog lung trematodes have been used as skin-testing antigens in schistosomiasis (*9*), antibodies could not be detected by hemagglutination after immunization. The hemagglutination test for this series of serums was more sensitive than the CHR (Cercarienhüllen Reaktion of Vogel and Minning, *10*) in detecting antibody; with one exception (spleen-egg antiserum), they were less sensitive than the miracidium immobilization test (*8*).

In Table 2, the titers of serums for agglutination of living cercariae of *S. mansoni* are compared with hemagglutination titers. *In vitro* agglutination tests with cercariae of *S. mansoni* were made in nine-depression Pyrex spot plates. Into each depression 0.5 ml of each serum dilution, plus 1 drop of Penicillin g (4000 units/ml), 1 drop of Streptomycin sulfate (0.5 mg/ml), and 1 drop of concentrated cercariae were added. Serums were diluted with 10-percent Ringer's solution, and control tests were made simultaneously with the diluent. Spot plates were placed in a moist chamber at room temperature (24° to 26°C), and readings were made the following day. Except for the chicken and pig, hemagglutination titers were much more sensitive.

As is noted earlier, the inactivated serums of several normal mammals agglutinated living schistosome cercariae. These serums were not titrated at this time. Approximately 1 year later, after these serums had been thawed and frozen many times, the normal and immune serums of the horse, cow, goat, and pig were retested. Agglutinins in normal serums were observed only in the horse, at a titer of 1:8, and in all immune serums at the same or higher titer (Table 2). The agglutination of cercariae in normal serum is probably caused by a nonspecific agglutinin, since all normal serums were negative by hemagglutination.

The hemagglutination titer obtained from day to day for a single serum may vary more than twofold. Fifteen hemagglutination tests of a single immune horse serum were made over a period of several months. Except for the first two tests where the titer dropped from 1:12560 to 1:320, the titers of 12 other tests fluctuated between 1:640 and 1:2560 (three tubes). The average titer for all tests was 1:1280. This titer was obtained in six of the 15 tests. Experiments with cells of different sheep, batches of normal rabbit serum, and lots of tannic acid by various manufacturers indicated that these variables were not responsible for fluctuation in the titer. The sensitivity of the test may vary with the solubility of the cercarial antigen used to coat the sheep red cells, since several batches of cercarial antigen were used. Work is in progress to obtain a soluble cercarial fraction that may be superior to the present antigen, and preliminary hemagglutination studies with human serums are being conducted.

Table 2. Comparison of agglutination and hemagglutination titers in animals immunized with cercariae of *S. mansoni*.

Host	No. of cercariae injected	Agglutination titer	Hemagglutination titer
Chicken	6,000	1:64	1:80
Cow	40,500	1:256	1:1280
Goat	26,000	1:64	1:5120
Horse	40,500	1:32	1:1280
Pig	25,000	1:128	1:320
Rabbit	18,000	1:32	1:160

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References and Notes

1. S. V. Boyden, *J. Exptl. Med.* **93**, 107 (1951).
2. A. B. Stavitsky, *J. Immunol.* **72**, 360 (1954).
3. C. Liu and F. B. Bang, *Soc. Exptl. Biol. Med.* **74**, 68 (1950).
4. O. D. Standen, *J. Helminthol.* **26**, 25 (1952).
5. M. A. Stirewalt and A. S. Evans, *Exptl. Parasitol.* **4**, 123 (1955).
6. D. M. Levine and I. G. Kagan, *J. Parasitol.* (suppl.) **40**, 36 (1954).
7. This investigation was supported by a research grant (G-4109) from the National Microbiological Institute, National Institutes of Health, U.S. Public Health Service.
8. I. G. Kagan, *Exptl. Parasitol*, in press.
9. J. T. Culbertson and H. M. Rose, *Am. J. Hyg.* **36**, 311 (1942).
10. H. Vogel and W. Minning, *Zentr. Bakteriol. Abt. 1 Orig.* **153**, 91 (1949).

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Effect of DDT on Enzymatic Oxidation and Phosphorylation

Despite numerous attempts to elucidate the mode of action of DDT [2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane] on adult houseflies, it must be recognized that this phenomenon has not yet been explained. Sacktor (*1*) reported that DDT inhibited cytochrome oxidase of houseflies, and suggested (*2*) that one of the explanations for the resistance of adult flies to the insecticide might be the greater oxidase activity developed in resistant strains. Recently Morrison and Brown (*3*) summarized much of the literature on this subject and reported DDT inhibition of cytochrome oxidase from American cockroaches. In the course of our search for DDT-sensitive reactions of adult houseflies we have discovered an effect of DDT that appears to be different from any reported previously (*4*). This effect consists of DDT-inhibition of oxidation of citric acid cycle intermediates, and of oxidative phosphorylation, when oxidation and phos-

phorylation are catalyzed by subcell particles from adult houseflies. The inhibition occurs at concentrations of DDT at which cytochrome oxidase appears to be unaffected.

Two hundred and fifty well-fed houseflies (*5*), *Musca domestica,* of mixed sex, that had been previously anesthetized by chilling in the cold room, were gently ground for 2 min in a mortar with 10 ml of homogenizing medium. The homogenizing medium was adjusted to *p*H 7.4 and contained 0.1*M* potassium phosphate, 0.25*M* sucrose, and 4 μmoles/ml each of pyruvate, citrate, α-ketoglutarate, succinate, and malate, which were added as preservatives of the particles. The resulting brei and a subsequent 5-ml rinse was squeezed through 8 thicknesses of No. 40 cheesecloth into a 15-ml plastic centrifuge cup. Immediately after this, the cellular debris and chitin were removed by sedimentation at 460 *g* in a refrigerated Servall centrifuge for 3 min. The particulate fraction was separated from the decanted low-speed supernatant by centrifuging at 12,800 *g* for 5 min. The particles were suspended in 5 ml of buffer and resedimented at 12,800 *g* for 5 min. The washed fraction was dispersed in 8.5 ml of homogenizing medium for addition to Warburg flasks. All equipment and solutions were prechilled in ice before use, and all preparatory steps, other than centrifugation, were conducted in a cold room. Microscopic examination of the particulate preparations showed a uniform field containing small particles that were stained with Janus green B. It is to be noted that when ascorbate was used as the principal substrate, each flask also contained 1 μmole of each of the substrates included in the homogenizing medium.

It may be seen in Table 1 that inhibition of oxidation of a substrate mixture, and of oxidative phosphorylation, was virtually complete at $5 \times 10^{-5}M$ (17.7 ppm) DDT and that an appreciable effect occurred at $5 \times 10^{-6}M$ (1.77 ppm). It was demonstrated in numerous other tests that the particulate preparations catalyzed oxidation of each of the substrates used in these experiments. Therefore, the data indicate that DDT inhibits, directly or indirectly, oxidation of every compound included as substrate.

To determine whether the effect noted was dependent upon inhibition of cytochrome oxidase, the oxidation of citric acid cycle intermediates and the oxidation of ascorbic acid were compared with respect to their susceptibility to DDT inhibition. Experiments not reported here demonstrated that oxygen uptake in this system reached a maximum when 50 to 100 μmoles of sodium ascorbate was added to each flask and when the concentration of added cytochrome *c* was $8 \times 10^{-5}M$. These concentrations were therefore used for the experiment reported in Table 2. It is apparent from the data in Table 2 that at least one-half of the ascorbate was oxidized through the cytochrome system, and it is reasonable to assume that most of it was so oxidized. Yet, under conditions in which the cytochrome system was loaded to a maximum, using ascorbate as the electron donor, no inhibition of oxidation was noted. In the same experiment, using the same particulate preparation, confirmation was obtained for DDT-inhibition of the oxidation of citric acid cycle substrates and of oxidative phosphorylation. In all of the experiments reported, this inhibition occurred in the presence or absence of added cytochrome *c*.

It does not appear that the DDT effects noted can be explained on the basis of an inhibition of cytochrome oxidase. Apparently DDT inhibits some other reaction involved in oxidation via the citric acid cycle; this occurs at DDT concentrations far lower than those at which cytochrome oxidase seems to be affected. Further work is obviously required to explain completely the data reported.

Table 2. Comparison of effect of DDT on ascorbate oxidation with effects on oxidation of citric acid cycle intermediates and phosphorylation by subcell particles from houseflies.* TCA denotes equimolar mixture of pyruvate, citrate, α-ketoglutarate, succinate, and malate.

Substrate (μmoles)	Added cytochrome *c* conc. (*M*)	DDT conc. (*M*)	O_2 uptake (μg atoms O_2/hr)	Phosphorylation (μmoles P/hr)
55 TCA‡	0		12.5	25
55 TCA‡	0	10^{-4}	1.5	− 3
55 TCA‡	8×10^{-5}		16.2	31
55 TCA‡	8×10^{-5}	10^{-4}	4.1	0
100 ascorbate + 5 TCA†	0		15.1	
100 ascorbate + 5 TCA†	8×10^{-5}		30.9	
100 ascorbate + 5 TCA†	8×10^{-5}	10^{-4}	31.3	
5 TCA†	0		2.8	
5 TCA†	0	10^{-4}	− 0.6	
5 TCA†	8×10^{-5}		2.5	
5 TCA†	8×10^{-5}	10^{-4}	− 0.9	

* Except as noted in the table, the additions to the flasks were the same as those in Table 1.
‡ Each value represents results from one Warburg vessel.
† Each value represents the average of results from two Warburg vessels.

Table 1. Effect of DDT on oxidation of citric acid cycle intermediates and on phosphorylation by subcell particles from houseflies.*

Ethanol-DDT concentration	Citric acid cycle oxidation (μg atoms O_2/hr)		Phosphorylation (μmoles P/hr)	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2
Control	13.8	12.6	34	30
Ethanol, 0.316 percent	13.0	12.3	38	30
DDT, $1 \times 10^{-4}M$ (35.5 ppm)	1.5	1.5	0	1.5
DDT, $5 \times 10^{-5}M$ (17.7 ppm)	1.0	2.0	0	2.0
DDT, $1 \times 10^{-5}M$ (3.55 ppm)	8.0	9.5	18	22
DDT, $5 \times 10^{-6}M$ (1.77 ppm)	10.0	10.5	22	26
DDT, $2.5 \times 10^{-6}M$ (0.89 ppm)	12.0	11.7	35	29
DDT, $1 \times 10^{-6}M$ (0.355 ppm)	12.3	12.1	32	30

* Each value represents the average of results from two Warburg vessels. The reaction was carried out in a final volume of 3.0 ml containing 200 μmoles potassium phosphate, 175 μmoles tris buffer (Sigma 7–9), 500 μmoles sucrose, 0.06 μmoles cytochrome *c*, 10 μmoles $MgSO_4$, 10 μmoles $ATPNa_2$ (Sigma), 6 mg plasma albumin (Armour), 5 mg hexokinase (Pabst), 200 μmoles glucose, and 11 μmoles each of pyruvate, citrate, α-ketoglutarate, succinate, and malate as sodium salts; 0.25 ml of the enzyme preparation was added to each flask. Final *p*H 7.3 to 7.4. Centerwell contained 0.2 ml 20 percent KOH. When DDT was added (in alcohol solutions), the final ethanol concentration was 0.316 percent.

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References and Notes

1. B. Sacktor, "The effect of DDT and methoxychlor on cytochrome oxidase in two strains of houseflies, *Musca domestica* L.," unpublished thesis, Rutgers University, 1949.
2. ———, *J. Econ. Entomol.* 43, 832 (1950).
3. P. E. Morrison and A. W. A. Brown, *ibid.* 47, 723 (1954).
4. Technical Paper No. 906, Oregon Agricultural Experiment Station. This investigation was supported in part by the Research and Development Division, Office of the Surgeon General, Department of the Army, under contract No. DA 49-007-MD-426.
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