very similar lesions in human SLE. What originally initiates such a process in the intact animal remains to be elucidated.

Summary. Autoimmune responses to liver damage by CCl_4 were induced in mice of three inbred strains, and tested for by complement fixation with liver homogenate. In 1-month-old mice, but not in adults, large strain differences were found: NZB having the highest titers, Balb/c intermediate, and C57Bl the lowest; NZB mice also developed more immunofluorescent staining of the kidneys after CCl_4. These results may have some bearing on the pathogenesis of the spontaneous autoimmune lesions in the NZB strain.

1. McDevitt, H. O. and Sela, M., J. Exptl. Med. **122**, 517 (1965).
2. Playfair, J. H. L., Immunology **15**, 35 (1968).
3. Bielschowsky, M. B., Helyer, B. J., and Howie, J. B., Proc. Univ. Otago Med. School **37**, 9 (1959).
4. Lambert, P. H. and Dixon, F. J., J. Exptl. Med. **127**, 507 (1968).
5. Weir, D. M., Immunology **6**, 581 (1963).
6. Miller, J. F. A. P., Proc. Roy. Soc. B. (London), Ser. **156**, 415 (1962).
7. Yunis, E. F., Hong, R., Crewe, M. A., Martinez, C., Cornelius, E., and Good, R. A., J. Exptl. Med. **125**, 947 (1967).
8. Pinckard, R. N. and Weir, D. M., Clin. Exptl. Immunol. **1**, 33 (1966).
9. Marshall, J. D., Eveland, C. E., and Smith, C. W., Proc. Soc. Exptl. Biol. **98**, 898 (1958).
10. Sinclair, N. R., Nature **208**, 1104 (1965).

Received Oct. 22, 1968. P.S.E.B.M., 1969, Vol. 131.

Hematological Effects of Apholate on Channel Catfish *(Ictalurus punctatus)* (33819)

CHARLES L. DODGEN AND SUE SULLIVAN
(Introduced by F. R. Blood)
Department of Biochemistry, University of Mississippi Medical Center, Jackson, Mississippi 39216

There have been many reports concerning the toxicological effects of the alkylating agent, apholate (2,2,4,4,6,6-hexahydro-2,2,4,4,6,6-hexakis (1-aziridinyl)-1,3,5,2,4,-6-triazatriphosphorine) in insects. Toxicological studies have been conducted in cattle (1), sheep (2), rats (3), mice (4), and fowls (5,6), but work with fish has been limited (7).

The delayed lethal syndrome resulting from treatment of animals with alkylating agents was reported by several workers, but the actual cause of death is not known (8). The present paper describes the hematological effects of oral administration of apholate to channel catfish (*Ictalurus punctatus*), and the delay in lethality, even with massive doses.

Materials and Methods. Channel catfish fingerlings, weighing 5–20 g, were maintained at 22° in 5 or 10-gal aquariums 10–14 days before, and during, experimentation. Single oral doses of apholate (crystallized from ethyl acetate) were administered in no. 5 gelatin capsules.

In the study of hematological changes produced by apholate, total erythrocyte and leukocyte counts were made by the method of Hesser (9), and differential leukocyte counts were made on thin smears stained with Wright's stain, using the classification of Jakowska (10). The blood was obtained by cardiac puncture using heparin as anticoagulant. Preliminary (normal) counts were made on 90 animals, and 2 weeks were allowed for recovery from cardiac puncture before treatment. Individual fish were marked for identification by small notches in the caudal fin, so that cell counts on each animal could be compared. The animals were then divided into three groups at random; 30 controls received empty capsules, 30 animals received 200 mg of apholate/kg; and 30 animals re-

TABLE I. Erythrocyte, Leukocyte, and Differential White Cell Counts of Normal Channel Catfish.

	Cells/mm³ (mean ± SE)
Erythrocytes	2.23 ± 0.05[a]
Total leukocytes	146.3 ± 8.3
Lymphocytes	105.7 ± 2.2
Thrombocytes	34.2 ± 2.2
Monocytes	0.4 ± 0.1
Neutrophils	3.7 ± 0.6
Hemocytoblasts	1.6 ± 0.2
Macrophages	0.4 ± 0.1
Eosinophils	0.3 ± 0.1

[a] Millions; all other counts in thousands.

ceived 400 mg of apholate/ kg. At 3, 6, and 9 days after treatment, blood samples were obtained again from 10 animals from each group. The animals were sacrificed and sexed by microscopic examination of the gonads. Significance of changes in blood cell counts was tested by ranking.

For the determination of the median lethal dose of apholate in channel catfish, the compound was administered to six groups of 10 animals each, at levels of 80–180 mg/kg of body weight. Two groups of 10 animals each were maintained as controls, and all animals were fasted during the experiment. The LD_{50} was determined by probit analysis.

Results and Discussion. The mean values for the blood cell counts of 90 normal channel catfish are given in Table I. As shown in Table I, lymphocytes and thrombocytes consitute approximately 95% of the total leukocyte count. Since the number of other leukocytes was quite low and variable, they could not be included in the statistical analysis of the differential counts. The changes in erythrocyte, total leukocyte, thrombocyte, and lymphocyte counts of treated animals are presented in Table II. The data in Table II represent, within each group of controls (Omg/kg) and apholate-treated animals (200 or 400 mg/kg), the mean changes (from initial, normal counts) due to treatment, with time. The changes in erythrocyte counts of animals receiving apholate at both levels were not significantly different from those of control animals. However, most groups showed a slight decrease in erythrocytes during the period between the first and second counts, indicating the desirability for each animal serving as its own control. The total leukocyte counts were significantly lower in both groups of apholate-treated fish than in controls at 6 and 9 days after treatment. The effect of apholate upon the lymphocytes was much greater than on the thrombocytes. In fact, there was no significant change in thrombocytes except at the higher dose of apholate. This difference, as well as the rapidity of all changes, is at least as striking as results obtained after administration of alkylating agents to mammals (8).

The lower dose used in the above experiments approximates the LD_{50} of 191 mg/kg, determined at 14 days post-treatment. The LD_{50} decreases to 124 mg/kg at 30 days, but one of the most interesting findings was that no deaths occurred in less than 8 days, regardless of dose. In another series of fish receiving as much as 2000 mg/kg, no animals died before the eighth day, compared to

TABLE II. Changes in Blood Cell Count after Treatment with Apholate.

Treatment (mg/kg)	Mean percentage of normal level[b]			
	Erythrocytes	Total leukocytes	Thrombocytes	Lymphocytes
3 Days post-treatment				
0	92.1	75.8	92.1[a]	77.0
200	95.1	69.8	192.8	66.0
400	86.1	53.4	82.7	42.5[c]
6 Days post-treatment				
0	93.2	103.8	215.3	103.2
200	93.6	55.1[d]	102.4[a]	50.8[e]
400	100.3	22.8[f]	26.2[a,f]	16.6[f]
9 Days post-treatment				
0	89.9	98.4	78.8	107.5
200	79.7	56.4[e]	56.1	62.6[e]
400	92.5	24.4[f]	101.0	17.7[f]

[a] Median used because of one extreme value in group.

[b] Level of significance determined by ranking, compared to "O" treatment.

[c] $.05 < p < .10$.

[d] $.02 < p < .05$.

[e] $.01 < p < .02$.

[f] $p < .01$.

3–7 days observed in mammals administered similar alkylating agents (8).

This lethality observed in the channel catfish might be accounted for by direct effects of apholate, or might be related to secondary bacterial infection. Six fish, treated with apholate for the hematology studies, died on the eighth and ninth days, and all these animals exhibited a heavy infection of the skin by gram-negative bacteria with sloughing of the epidermis. However, one other moribund animal, which had been treated with 400 mg of apholate/kg 8 days previously, showed no histological sign of infection. This fish did exhibit the sloughing of the epidermis, and the gross appearance of the skin was identical to that of infected fish.

In summary, it was shown that apholate has a profound effect in producing a lymphocytopenia in channel catfish, but less effect on the thrombocytes. The delayed death due to apholate poisoning is quite similar to that observed in mammals (8). Infection of the skin was present in all apholate-treated animals studied with one exception. This animal, however, did show the usual sloughing of the skin, but histological examination showed no evidence of infection. From these findings it appears that infection may contribute to death of apholate-treated channel catfish, but, as in mammals, the basic cause of death is unknown.

Apholate was graciously supplied by E. R. Squibb and Sons, New Brunswick, New Jersey. Channel catfish fingerlings were obtained from the National Fish Hatchery, Tupelo, Mississippi. The authors wish to thank Dr. Catherine Goetz for making the histological examinations. This work supported by USPHS grants numbers EF 00438, ES 00137, and CC 00271.

1. Khan, M. A., Can. J. Comp. Med. Vet. Sci. **27**, 233 (1963).

2. Younger, R. L. and Young, J. E., Am. J. Vet. Res. **24**, 659 (1963).

3. Gaines, T. B. and Kimbrough, R. D., Bull. World Health Organ. **31**, 737 (1964).

4. Ristich, S. S., Ratcliffe, R. H., and Perlman, D., J. Econ. Entomol. **58**, 929 (1965).

5. Sherman, M. and Herrick, R. B., Toxicol. Appl. Pharmacol. **9**, 279 (1966).

6. Shellenberger, T. E., Skinner, W. A., and Lee, J. M., Toxicol. Appl. Pharmacol. **10**, 69 (1967).

7. Eisler, R., Progressive Fish Culturist **28**, 154 (1966).

8. Hayes, W. J., Jr., Bull. World Health Organ. **31**, 721 (1964).

9. Hesser, E. F., Progressive Fish Culturist 22, 164 (1960).

10. Jakowska, S., Rev. Hematol. **11**, 519 (1956).

Received Oct. 28, 1968. P.S.E.B.M., 1969, Vol. 131.