may critically reduce blood flow to important vascular beds. The use of phenoxybenzamine in treatment of shock is based on the assumption that blockade of the vascular constrictor response to adrenergic agents released in shock may contribute to survival(7,9,11). If phenoxybenzamine prevents vasoconstriction imposed by humoral vasopressors and consequently increases blood flow in shock, the present study indicates that this increased flow to critical vascular beds does not promote survival.

Summary. The survival value of both pre- and post-treatment with phenoxybenzamine was evaluated in dogs given lethal injections of *E. coli* endotoxin. Pretreatment with either 1 or 5 mg/kg phenoxybenzamine did not promote survival after injections of endotoxin. Post-treatment with phenoxybenzamine and plasma was also of no therapeutic value in promoting survival after lethal injections of endotoxin.

1. Weil, M. H., Miller, B. S., *J. Lab. Clin. Med.*, 1961, v57, 683.

2. Lillehei, R. C., MacLean, L. D., *A.M.A. Arch Surg.*, 1959, v78, 464.

3. Weil, M. H., Sudrann, R. B., Shubin, H., *Calif. Med.*, 1962, v96, 86.

4. Nickerson, M., *J. Mich. Med. Assn.*, 1955, v54, 45.

5. Zweifach, B. W., Thomas, L., *J. Exp. Med.*, 1957, v106, 385.

6. Yard, A. C., Nickerson, M., *Fed. Proc.*, 1956, v15, 502.

7. Gourzis, J. T., Hollenberg, M. W., Nickerson, M., *J. Exp. Med.*, 1961, v114, 593.

8. Ravdin, I. S., Hardy, J. D., Alican, F., *Am. J. Med. Sci.*, 1962, v244, 237.

9. Longerbeam, J. K., Lillehei, R. C., Scott, W. R., Rosenberg, J. C., *J. Am. Med. Assn.*, 1962, v181, 878.

10. Vick, J. A., LaFave, J. W., MacLean, L. D., *Surgery*, 1963, v54, 78.

11. Nickerson, M., Gourzis, J. T., *J. Trauma*, 1962, v2, 309.

12. Lillehei, R. C., Longerbeam, J. K., Bloch, J. H., *Am. J. Cardiol.*, 1963, v12, 599.

13. Nickerson, M., *Fed. Proc.*, 1960, v20, Part III, 98.

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Enhanced Antibody Formation in Experimental Acute and Chronic Liver Injury Produced by Carbon Tetrachloride or Allyl Alcohol.* (29451)

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In human hepatic diseases the stimulation of mesenchyma has been demonstrated by morphological techniques(1). This stimulation also involves immunologically competent cells which are included in the reticuloendothelial system(2,3). Although serum elevation of gamma globulin is common, its antibody nature is still in question. After immunization of patients with liver disease, some observers(4,5) have found increased antibody production, but others(6) do not confirm this finding. Histological techniques have demonstrated stimulation of the immunocytic or protein-forming part of the reticuloendothelial system in animals with acute liver injury caused by administration of CCl_4(7), or in those with chronic hepatic damage after ethionine feeding(8), or after exposure to CCl_4(9).

Therefore, a study was attempted of the immune response in mice with acute and chronic hepatic injury, using serological and immunocytochemical techniques correlated with pathological investigations.

Materials and methods. A total of 265 male Swiss-Webster mice, weighing an average of 30 g each, were divided into several groups (Tables I and II). CCl_4 was injected subcutaneously in the amount of 0.03 ml†,

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† Unless so stated, CCl_4 was not suspended in olive oil.

TABLE I. Effect of Antigen Concentration (Groups A-F) and Temporal Relationship Between CCl_4 and Immunization (Groups G-J) on Agglutinin Levels of Mice Exposed Twice to CCl_4 and Controls.

Group	No. mice	Injection*	Immunization Antigen†	Concentration (ml%)	Mean antibody titer (Log_2 titer)‡	S±§	P
A	10	None	RRBC	.05	.00		
B	10	CCl_4	"	.05	1.02	.41	<.05
C	9	O.O.‖	"	.1	.26	.24	
D	10	CCl_4–O.O.¶	"	.1	2.76	1.09	<.05
E	24	O.O.‖	"	5	4.71	.52	
F	23	CCl_4–O.O.¶	"	5	7.02	.61	<.01
G	19	CCl_4**	SRBC	.05	1.63	.45	>.20
H	19	CCl_4	"	.05	2.64	.49	<.01
I	18	PS	"	.05	.83	.29	
J	10	PS	none		.00		

* Injections were given 6 hr before immunization, except Group G. They were repeated 3 days after immunization.
† RRBC = rat red blood cells; SRBC = sheep red blood cells.
‡ One was added to each observation to eliminate the difficulty of taking the Log_0.
§ S = standard error of mean.
‖ O.O. = olive oil, .06 ml each injection.
¶ CCl_4–O.O. = CCl_4 dissolved in olive oil 1:1; .06 ml each injection.
** CCl_4 was given 6 hr after immunization.

while 0.6 ml of a 0.3% solution of allyl alcohol (AA) in physiological saline (PS) was injected intraperitoneally. The control groups consisted of 10 nonimmunized and untreated mice and of 15 mice receiving CCl_4 biweekly for 40 days.

The findings of the hepatic changes in the liver after injections of CCl_4 or AA have previously been described(10,11) and were confirmed in this study.

For immunization, intraperitoneal injections were made of either 1 ml of thrice-washed rat or sheep red cells or 0.3 ml of sterile horse serum (Pentex). Blood was collected from the heart 7 days after the last injection, or (in Groups R and S) at the end of the experiment. Then the animals were sacrificed and their body and spleen weights were recorded. Agglutinin titers were determined by Stimpfling's method(12), and the levels of anti-horse serum antibodies were established by a semimicro precipitin technique(13). The reciprocal of the highest serum dilution still giving a positive reaction was considered to be the serum titer, and for the purpose of statistical analysis, the titer values were converted to log_2 titers.

Blocks from liver, spleen, mesenteric, hepatic and axillary lymph nodes were quickly frozen in dry ice isopentane and stored at −20°C until processed for immuno-fluorescence, and sections were cut,

TABLE II. Effect of Repeated Administration of CCl_4 or Allyl Alcohol (AA) on Circulation Antibodies Against Horse Serum.

Group	No. of mice	Treatment (biweekly)	Duration (days): Experiment	Treatment	Immunization*	Mean antibody titer (Log_2 titer)	S±†	P	Mortality (%)
K	13	PS	40	40	40	3.08	.35		0
L	14	CCl_4	40	40	40	5.64	.29	<.001	6.66
M	6	CCl_4	100	first 60	last 40	5.17	.54	<.01	40.00
N	11	AA	40	40	40	4.05	.03	<.01	8.33
O	9	PS	100	100	100	4.38	.03		10.00
P	7	CCl_4	100	100	100	6.00	.02	<.01	41.66
Q	10	CCl_4	100	first 60	100	5.83	.02	<.001	16.66
R	11	CCl_4	100	first 60	first 60	3.55	.31		8.33
S	7	CCl_4	100	100	first 60	5.43	.42	<.01	41.66

* Weekly injection of .3 ml of horse serum.
† S = standard error of mean.

stained and observed as previously described (14). Both direct and indirect techniques were used to detect antibodies, employing either horse serum that had been fluoresceinated or horse serum followed by fluoresceinated anti-horse serum prepared in rabbits. Mice gamma globulin was visualized by a fluorescein-labeled antiserum against mouse gamma globulin, and was separated with a DEAE cellulose column(15).

Results. Treatment with CCl_4 enhances the agglutinin titer in mice immunized with 1 ml of 0.05, 0.1 or 0.5 ml % suspension of rat cells (Table I, Groups A-F). The agglutinin response was highest in those animals which were given their first dose of CCl_4 six hours before immunization (Table I, Groups G-J).

Weekly administration of horse serum, combined with bi-weekly injections of CCl_4 for periods of 40 or 100 days, produced a rise in the specific antibody titers (Table II, Groups K-L and O-P). The titers rose also if the CCl_4 injections were discontinued (Table II, Groups M and Q), or if CCl_4 treatment was prolonged for 40 days after immunization (Table II, Groups R-S). The antibody level was greater also in those mice treated with allyl alcohol for 40 days (Table II, Group N). Animals which were immunized and repeatedly injected with CCl_4 for 60 or more days had a higher mortality from anaphylaxis than did either the control animals which had only been immunized or those mice on which the CCl_4 treatment or the immunization had been discontinued (Table II).

The spleens of the nonimmunized mice treated with CCl_4 for 40 days weighed more (mg 189 $\pm$ 20) than did those of the controls (mg 113 $\pm$ 23), while the mice injected with CCl_4 and immunized for 40 and 100 days exhibited large spleens (mg 404 $\pm$ 165 and 603 $\pm$ 140 respectively). The difference between these spleens and those of the corresponding control animals which had only been immunized for 40 and 100 days (mg 308 $\pm$ 158 and 259 $\pm$ 80 respectively) was statistically significant, with a $P < 0.001$. There was no difference between the body weights of the various groups and those of the control animals.

The lymphoid tissue of CCl_4- and AA-injected mice presented not only conspicuous reticuloendothelial proliferation with increased macrophages and autofluorescent lipofuscin, but also mature and immature plasma cells containing gamma globulin. Immunofluorescent studies of the mice treated with horse serum and CCl_4 showed numerous cells containing antibodies in the spleen and lymph nodes, particularly the ones regional to the liver (Fig. 1 and 2). No cells containing antibodies were found in their livers. The lymphoid systems of the control mice treated only with horse serum showed only a few scattered cells containing antibodies.

Discussion. Both acute and prolonged administration of CCl_4 to mice raises the antibody levels. In acute CCl_4 intoxication, high titers of agglutinins can be seen in animals which have received various amounts of antigen. Furthermore, the time relationship between administration of CCl_4 and immunization is important because the increase in agglutinin production is greater when antigenic stimulation follows CCl_4 injection.

The increase in antibody level is more evident when the CCl_4 injection is associated with repeated antigen injections, and the prolonged administration of CCl_4 seems to have an enhancing effect regardless of its relationship to immunization. When the mice were given CCl_4 either before or during the immunization, an increased antibody formation resulted, and the antibody level remained high if the intoxication was not discontinued. Other hepatotoxic drugs, such as allyl alcohol, have the same antibody-enhancing effect as CCl_4. The increased susceptibility to anaphylactic shock, found in mice treated with CCl_4 for 60 or more days, also reflects the high level of circulating antibodies.

Different schedules of CCl_4 and antigen injections will account for reported findings of "no effect"(16,17) or block in antibody formation(18) in rabbits and guinea pigs treated with CCl_4. Species differences can also be responsible. In one brief report(19) however, mention was made that CCl_4 increased antibody production in rats, and in

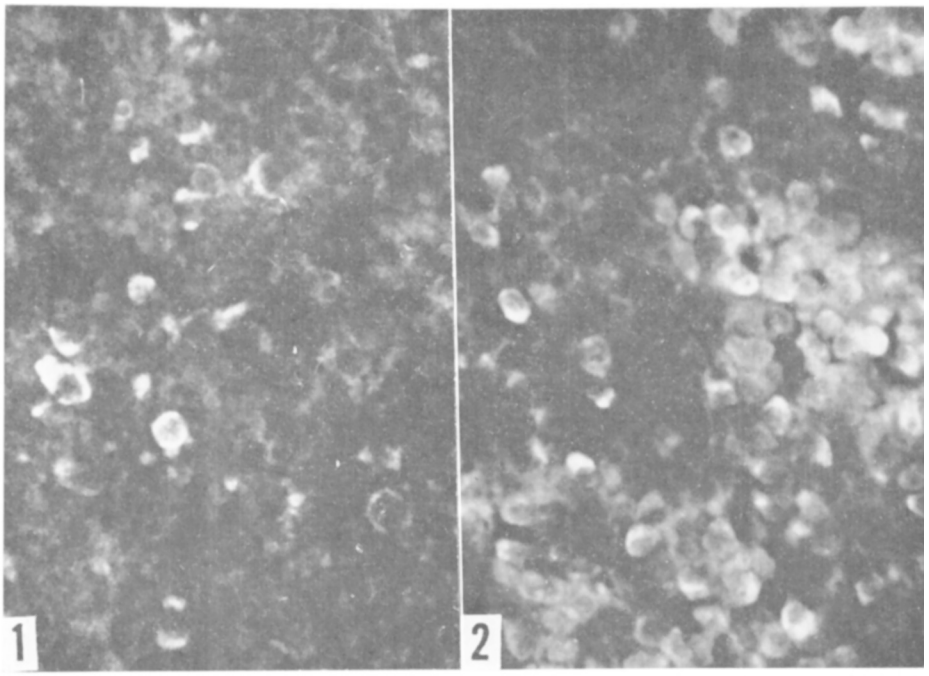

FIG. 1. Hepatic lymph node from a mouse immunized with horse serum for 40 days. Note few scattered cells containing specific antibodies (section treated with horse serum and then fluorescein-labelled antihorse serum, × 400).

FIG. 2. Hepatic lymph node from a mouse treated with CCl_4 and immunized with horse serum for 40 days. Clusters of cells containing specific antibodies are seen (sections treated as in Fig. 1, × 400).

another, CCl_4 was said to elicit the formation of autoantibodies in rats(20).

Several mechanisms may account for the influence of hepatotoxic agents on antibody production: *i.e.*, 1) they may act directly on the reticuloendothelial system, activating the antibody-forming cells; or 2) they may alter phagocytosis or hepatic blood flow, improving utilization of the injected antigen; or 3) they may act indirectly through a hepatic mechanism, e.g., liver cell breakdown products may act as an adjuvant, since nucleoprotein(21) or nucleoprotein derivatives(22) enhance antibody production.

There is agreement between the offered explanation of an exuberant and progressive proliferation of immunocytes in the spleen and lymph nodes caused, directly or indirectly, by the hepatotoxic drugs, and the immunocytochemical observations. After an antigenic stimulation, the lymphoid systems of animals treated with hepatotoxic drugs contain many more cells forming antibodies. Thus a quantitative alteration of the immunological reactivity induced by an adjuvant effect of liver injury might play a role in the pathogenesis of some liver diseases.

Summary. Two injections of CCl_4 produce enhancement of agglutinins in mice receiving various amounts of heterologous red cells after the first injection of CCl_4. Prolonged administration of CCl_4 or allyl alcohol enhances antibody levels against horse serum, and increases susceptibility to anaphylactic shock. CCl_4 is effective whether given before or during immunization, or if continued after immunization. In CCl_4-treated mice, the weight of the spleen increases, and the lymphoid system exhibits proliferation of cells containing gamma globulin. In immunized mice treated with CCl_4 or allyl alcohol, these cells produce specific antibodies that indicate a quantitative alteration of the immunological reactivity. Liver injury seems to have an "adjuvant" effect.

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1. Schaffner, F., Barka, T., Popper, H., *Exp. Mol. Path.*, 1963, v2, 419.
2. McMichael, J., *J. Path. Bact.*, 1934, v39, 481.
3. Glagov, S., Kent, G., Popper, H., *Arch. Path.*, 1959, v67, 9.
4. Havens, W. P., Jr., Shaffer, J. M., Hopke, C. J., Jr., *J. Immunol.*, 1951, v67, 347.
5. Havens, W. P., Jr., Myerson, R. M., Klatchko, J., *New Engl. J. Med.*, 1957, v257, 637.
6. Cherrick, G. R., Pothier, L., Dufour, J. J., Sherlock, S., *ibid.*, 1959, v261, 240.
7. Wirtschafter, Z. T., DeMerrit, M. G., *Arch. Path.*, 1959, v67, 176.
8. Kent, G., Popper, H., Dubin, A., Bruce, C., *ibid.*, 1957, v64, 398.
9. Menon, T. B., *J. Path. Bact.*, 1938, v46, 521.
10. Cameron, G. R., DeSaram, G. S. W., *ibid.*, 1939, v40, 41.
11. Eger, W., *Virchows Arch. f. path. Anat.*, 1956, v328, 536.
12. Stimpfling, J. H., *Transpl. Bull.*, 1961, v27, 109.
13. Boyd, W. C., *Fundamentals of Immunology*. Interscience, N. Y., 1959, 3rd Ed., 660.
14. Paronetto, F., Rubin, E., Popper, H., *Lab. Invest.*, 1962, v11, 150.
15. Levy, H. B., Sober, H. S., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 250.
16. Hadnagy, D., Gyergyay, F., *Z. f. Immunitäts forsch. u. exper. Therap.*, 1958, v115, 357.
17. Rice, C. E., *J. Immunol.*, 1954, v73, 375.
18. Annenkov, G. A., *J. Microbiol. Epidem. Immunobiol.*, 1957, v28, 484.
19. Heuer, A., Pernis, B., Braun, W., *Bact. Proc.*, 1962, p.74 (abst.).
20. Weir, D. M., *Immunology*, 1963, v6, 581.
21. Merritt, K., Johnson, A. G., *Fed. Proc.*, 1964, v23, 139.
22. Taliaferro, W. H., Jaroslow, B. N., *J. Infect. Dis.*, 1960, v107, 341.

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Failure of RNA to Inhibit Rejection of Skin Homografts.* (29452)

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Ashley *et al*(1), Axelrod and Lowe(2) and Jolley, Hinshaw and Peterson(3) have reported that the systemic administration of ribonucleic acid (RNA) to experimental animals resulted in prolongation of skin homograft survival. Prior work from this laboratory(4) has demonstrated that incubation *in vitro* with lymphoid RNA may inhibit sensitized rabbit lymph node cells from manifesting transplantation immunity, as indicated by the inability of these cells to produce a "transfer reaction"(5).

It therefore appeared desirable to attempt to inhibit the rejection of skin homografts in rabbits by the infusion of lymphoid RNA *in vivo* and, in addition, to determine whether or not it was possible, in our laboratory, to confirm the work of Jolley and co-workers (3), who reported markedly prolonged survival of skin homografts in rabbits induced by repeated administration of homologous, liver RNA.

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Materials and methods. Purposely outbred New Zealand White rabbits were used as experimental animals. The rabbits weighed approximately 2 kg. The techniques of excision of spleen and lymph nodes and of full thickness skin grafting used in this laboratory have been described(4,6). In these experiments the skin grafts, approximately 1.5 cm square, were excised from and sewn in place on the rabbits' ears in all instances.

Extraction of RNA from rabbit tissue by the cold phenol method has also been described(4,6). The concentration of RNA in the preparations used in these experiments was determined by the optical density at 260 mμ in a Beckman model DU spectrophotometer.

The survival of skin homografts in the present experiments was considered to have ended when they showed unequivocal signs of rejection—eschar formation, infarction, loss of pliability, etc. Skin autografts were used as controls in all experiments. The autografts in all animals discussed below sur-