

The effect of chronic alcohol administration on the immune responsiveness of rats

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A series of studies were carried out to determine the effect of chronic alcohol administration on cellular and humoral immunity in the rat. Alcohol-treated rats received an alcohol liquid formula diet for 3 months, and this uniformly resulted in the development of a fatty liver. In alcohol-treated rats there was a delay in antibody production to both typhoid H and B. abortus antigens following a primary immunization. The peak titers obtained, however, were not significantly different from those of the control animals. The secondary response to typhoid H antigen was not affected. The expression of cutaneous delayed hypersensitivity to a potent skin sensitizing chemical, 2, 4-dinitrofluorobenzene, was also depressed in alcohol-treated rats. Animals receiving alcohol also had markedly atrophic thymuses and small spleens. The normal thymuses and adrenal glands found in control animals treated with ACTH injections indicate that the changes were not due to hyperactivity of the adrenal cortex in a stressful situation. It is postulated that the deleterious effects of alcohol on the immune system of rats may be due to alteration in either reticuloendothelial or thymic function.

Several clinical studies have indicated that alcoholic patients are unusually susceptible to severe infections and bacteremia.¹⁻⁴ Infections with gram-negative organisms, tuberculosis, and renal papillary necrosis seem to occur frequently in alcoholic patients.¹⁻⁵ In addition, the course of a pneumococcal infection may be especially severe in alcoholic patients.^{6, 7} The reasons for the increased incidence and severity of infections in these patients are not clear. It has been suggested that bacteremia is more common in patients with Laennec's cirrhosis than in normal persons because of an impaired filtering capacity of the diseased liver.⁴ It has also been proposed that recurrent infections in such patients may be due to decreased host defense resulting from decreased bone mar-

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row granulocytic reserve and a diminished granulocytic response.^{8, 9} There is preliminary evidence indicating that increased susceptibility to infections may be due to a depressive effect of alcohol on the phagocytic function of the reticulo-endothelial system.¹⁰

An increased level of serum globulins is a frequent finding in chronic liver disease¹¹ and in experimental liver injury.¹² In this connection, it has been reported that antibody production is actually increased in animals with experimentally induced liver disease¹³ and in human beings with cirrhosis associated with chronic alcoholism.¹⁴ However, the effect of alcohol per se on immunologic responsiveness has not been clearly defined.

In the present investigation, studies were carried out to determine the effect of chronic alcohol administration on cellular and humoral immunity in the rat. The data obtained indicated that chronic alcohol ingestion resulted in a delayed primary immune response to both typhoid H and *Brucella abortus* antigens and suppression of cutaneous delayed hypersensitivity to 2, 4-dinitrofluorobenzene (DNFB).

MATERIALS AND METHODS

Female Wistar rats* weighing 120 to 140 grams were separated into 3 groups, housed in individual cages, and given liquid diets in drinking tubes. All animals were maintained on liquid-formula diets for 10 weeks. Twenty-four control animals received a liquid-formula diet containing adequate quantities of carbohydrate, fat, amino acids, salts, and vitamins as described by Lieber, Jones, and DeCarli.¹⁵ Twenty-four alcohol-treated animals received an identical liquid formula diet, except that alcohol was substituted for 36 per cent of the carbohydrate calories and 20 per cent of the total caloric intake. Lieber and associates¹⁵ have demonstrated that this alcohol diet consistently leads to the development of a fatty liver within 24 days. Six ACTH-treated animals received a liquid-formula diet identical to that of the control animals and, in addition, were given injections of 10 units of zinc ACTH, subcutaneously, at weekly intervals. These animals served as controls for the stress situation that may have resulted from the chronic alcohol administration.

On Day 24 after initiation of the liquid-formula diets, all animals were immunized with typhoid H antigen† given in a total dose of 0.5 ml., 0.2 ml. into an inguinal area subcutaneously and 0.3 ml. intraperitoneally. On Day 52 second identical injections of typhoid H were given to each animal. On Day 38 each animal was immunized with 1.0 ml. of *Brucella abortus* antigen‡ (1×10^9 organisms per milliliter), 0.5 ml. in an inguinal area subcutaneously and 0.5 ml. intraperitoneally. Under light ether anesthesia, blood specimens were obtained from the femoral vein. Blood samples were drawn on Days 7, 14, 21, and 28, following primary immunization with typhoid H and *B. abortus*, and on Day 14 following the second injection of typhoid. The serum samples were frozen at -20° C. until agglutination tests were performed. Serum samples from

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each animal were coded and treated individually in a blind fashion. For typhoid H agglutinin determinations, twofold serial dilutions of 0.1 ml. of serum were made in saline from an initial 1:10 dilution in the first tube, followed by addition of 0.1 ml. of the typhoid H tube test antigen.* The reaction mixture was incubated at 37° C. for 2 hours and then read. Agglutinin titers were expressed as the last tube number showing a 2+ agglutination. A tube value of 1 was arbitrarily assigned to the initial tube containing a final 1:20 dilution. The actual serum dilution corresponding to a titer thus expressed can be calculated from the formula 10×2^n , where n = last tube number showing agglutination. For *B. abortus* agglutinin determinations, twofold serial dilutions of 0.1 ml. were made in saline, followed by addition of 0.1 ml. of the *B. abortus* antigen containing 1×10^9 organisms per milliliter. The reaction mixture was incubated at 37° C. for 2 hours and then read. Titers were expressed as the reciprocal of the last tube giving a 2+ agglutination. Statistical evaluation of the data was made by an analysis of variance and Student's *t* test.

On Day 26, 0.1 ml. of a 1 per cent solution of 2, 4-DNFB† in acetone was placed on a shaved area of the back of each animal. Three weeks later, one drop of the one per cent DNFB dilution was placed on a different site on the back. Readings for evidence of cutaneous delayed hypersensitivity were taken at 24, 48, and 72 hours, and the maximal reading was recorded. The presence of erythema was interpreted as a minimal (1+) reaction. Exudation and crusting were considered moderate (2+) reactions. Evidence of necrosis was interpreted as a severe (3+) reaction. Statistical evaluation of the data was performed with the use of chi square analysis.

All animals were bled while under light ether anesthesia. The spleen, thymus, and adrenal glands were removed, and all extraneous tissue was trimmed away. The organs were weighed, representative samples were fixed in 10 per cent buffered formalin, and sections were prepared for histological examination. The organ weights were expressed as milligrams per 100 grams of body weight, and statistical evaluation of the data was made with Student's *t* test.

RESULTS

Changes in typhoid H agglutinin titers for control and alcohol-treated animals are shown in Fig. 1. Fig. 2 shows data on the changes in *B. abortus* titers for the same control and alcohol groups. The antibody response to both antigens was delayed in the alcohol-treated group. The typhoid H agglutinin titer was significantly less than the control group at both 7 (1:40 versus 1:80, $p = < 0.01$) and 14 days (1:80 versus 1:320, $p = < 0.005$). The *B. abortus* agglutinin titer in the alcohol-treated animals was significantly less than that of the control group at 7 days (1:16 in the former and 1:64 in the latter group, $p = < 0.001$). The peak titers to both antigens were not significantly different in the 2 groups of animals. Similarly, the secondary response to typhoid H antigen did not differ significantly between the 2 groups.

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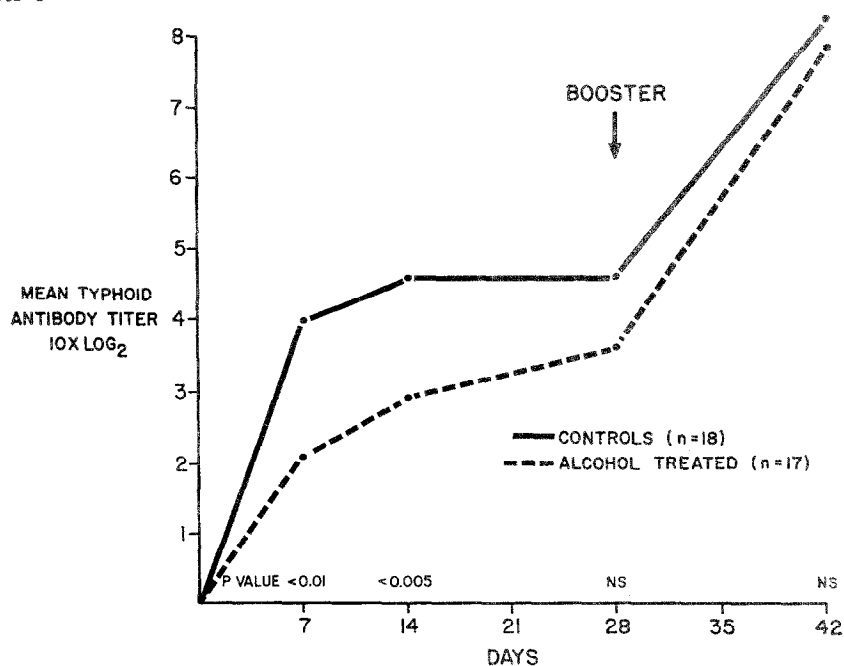


Fig. 1

Changes in typhoid H agglutinin titers for control and alcohol-treated animals immunized with *S. typhosa* in a dosage of 0.5 ml. on Day 24 and on Day 42.

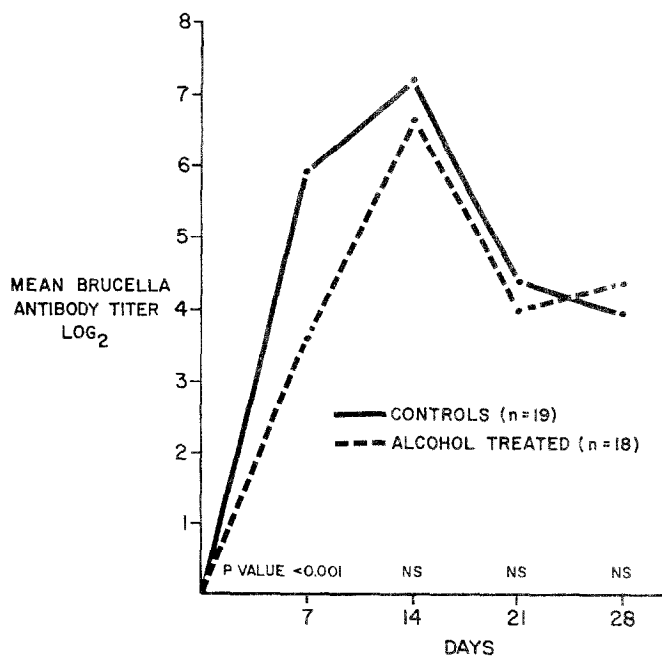


Fig. 2

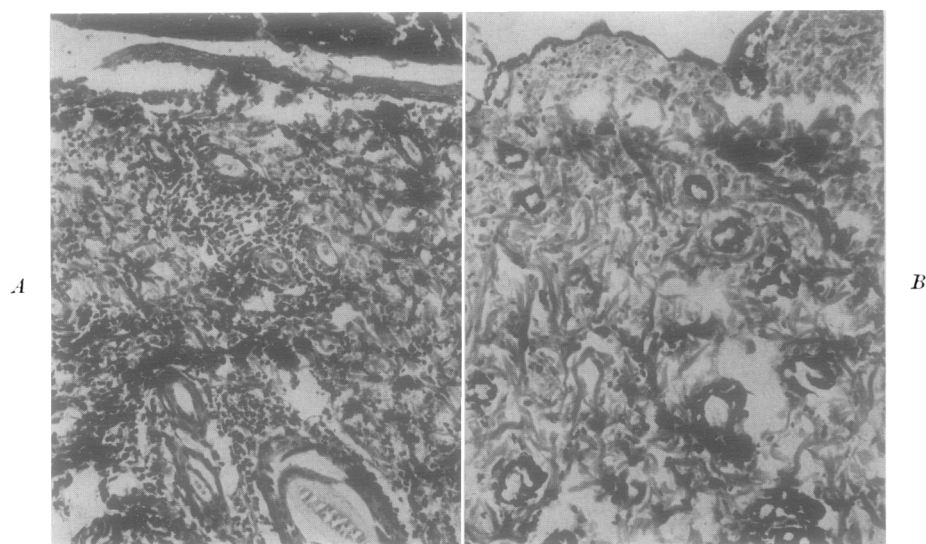
Changes in *B. abortus* agglutinin titers for control and alcohol-treated animals immunized with *B. abortus* in a dosage of 1.0 ml. (1×10^9 organisms per milliliter) on Day 38.

Table 1. *Effect of alcohol treatment on cutaneous delayed hypersensitivity to DNFB*

Group	Number of animals	Cutaneous response to DNFB				
		Neg.	1+	2+	3+	2+ or greater
Alcohol	16	6	7	3	0	$3 > p < 0.005$ $16 > \text{N.S.}^*$
Control	21	2	3	12	4	
ACTH	5	2	0	3	0	$3 > \text{N.S.}^*$

For details of DNFB methods, see text. Interpretation of skin lesions was as follows: 1+ = erythema; 2+ = erythema and crust formation; 3+ = ulceration.

*N.S. = no significant difference.

**Fig. 3**

Skin biopsy specimens from animals sensitized and tested with 1:100 2, 4-DNFB topically applied. *A*, Control animal showing infiltration of perivascular mononuclear and polymorphonuclear leukocytes in subcutaneous tissue with the presence of necrotic epidermis. *B*, Alcohol-treated animal showing only minimal infiltration of polymorphonuclear leukocytes with an intact epidermis. Hematoxylin and eosin $\times 170$.

Table I summarizes the response to DNFB challenge in the alcohol-treated and control groups. Only 3 of 16 alcohol-treated animals exhibited a 2+ or greater response as compared with 16 of 21 control animals ($p = < 0.005$). In Fig. 3 the histologic findings in biopsy specimens of the DNFB skin tests are compared in sections from alcohol-treated and control animals. The section from the control rat shows a severe reaction with perivascular mononuclear and polymorphonuclear infiltration in the subcutaneous tissue and a necrotic epidermis. By contrast, there is only minimal infiltration of polymorphonuclear leukocytes in the dermis of the alcohol-treated animal, and the epidermis is intact.

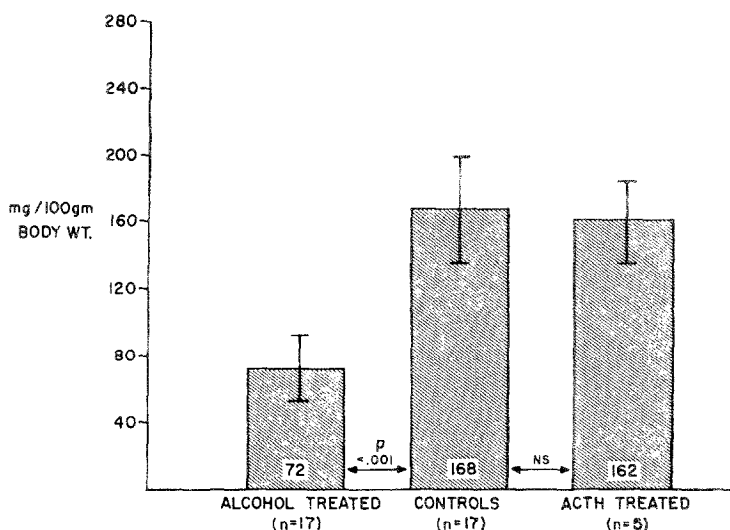
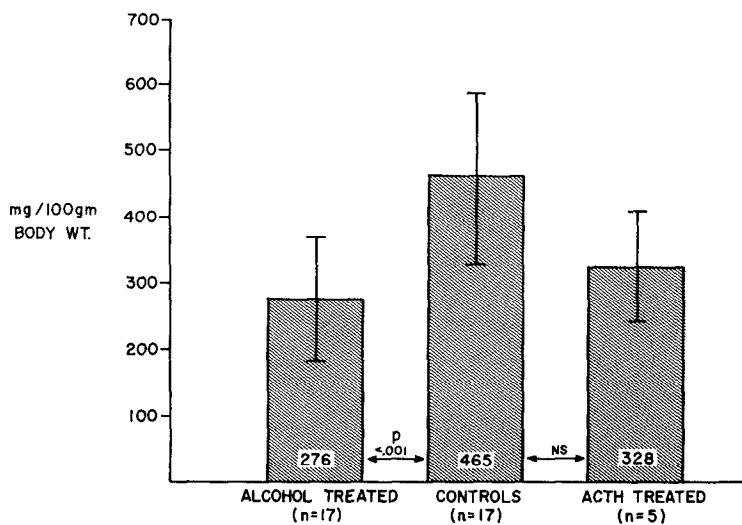


Fig. 4

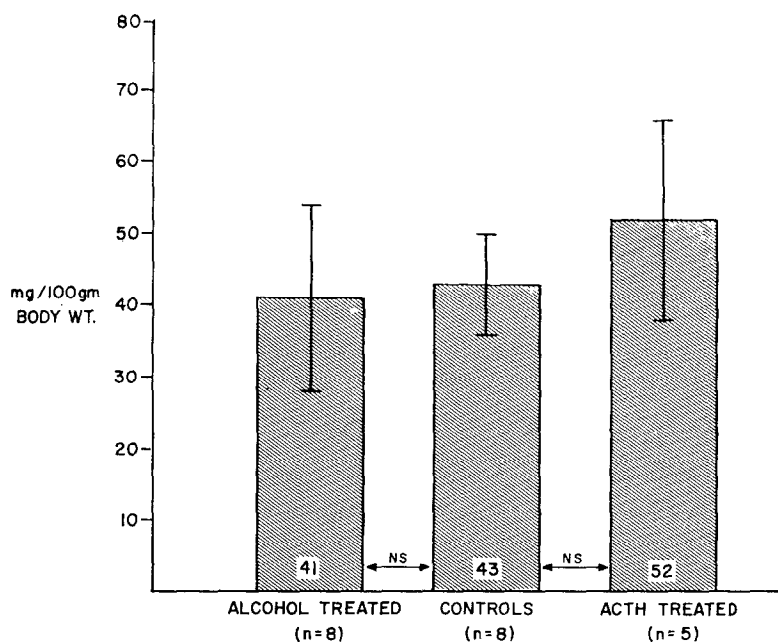
Mean weight of thymuses from control, alcohol-treated, and ACTH-treated animals determined at the end of the experiment (90 days). ACTH-treated animals received 10 units of zinc ACTH weekly.

Body weights of the alcohol-treated animals and the control animals did not differ significantly at the beginning of the immunization period (147 ± 6 [± 1 S.D.] grams versus 146 ± 7 grams) or at the time of exsanguination (199 ± 27 grams versus 218 ± 19 grams). There were no significant differences in the total white blood cell counts ($7,000 \pm 2,400$ versus $9,000 \pm 4,500$ per milliliter) or total lymphocyte counts ($5,000 \pm 2,200$ versus $4,800 \pm 2,900$ per milliliter) between control and alcohol-treated animals at the end of the study period.

The effects of chronic alcohol and ACTH administration on thymic weights are shown in Fig. 4. The mean weight of the thymus was 168 ± 32 milligrams per 100 grams of body weight in control animals. By contrast, the mean thymic weight in the alcohol-treated animals was 72 ± 20 milligrams per 100 grams of body weight. The thymuses from the alcohol-treated group were significantly smaller and atrophic when compared with those of the control group ($p = < 0.001$). The mean thymic weight of 162 ± 28 milligrams per 100 grams of body weight in animals receiving weekly ACTH injections was not significantly different from that of the control group. The splenic weights found in each of the study groups are shown in Fig. 5. The mean splenic weight of the alcohol-treated group (276 ± 93 milligrams per 100 grams of body weight) was significantly smaller ($p = < 0.001$) than the mean value of 465 ± 132 milligrams per 100 grams of body weight of the control group. The mean splenic weight of the ACTH-treated animals (328 ± 87 milligrams per 100 grams of body weight) was less than that found in the control group; however, this was not statistically significant. In Fig. 6 the adrenal weights of each of the study groups are shown. The mean adrenal weights were 41 ± 13 milligrams, 43 ± 7 milligrams, and 52 ± 14 milligrams per 100 grams of body

**Fig. 5**

Mean weight of spleens from control, alcohol-treated, and ACTH-treated animals.

**Fig. 6**

Mean weight of adrenal glands from control, alcohol-treated, and ACTH-treated animals.

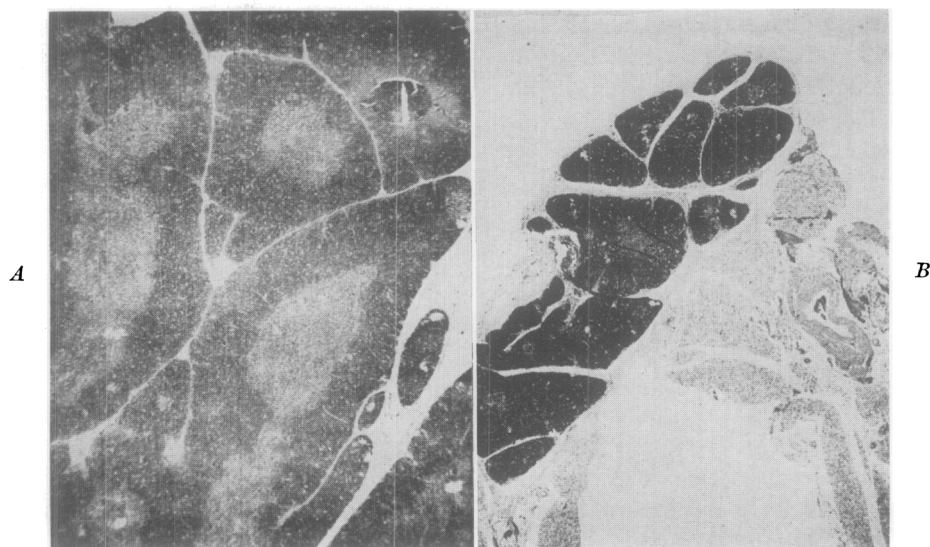


Fig. 7

A, Thymus from control animal showing well-defined cortex and medulla. *B*, Thymus from alcohol-treated animal showing marked atrophy with poorly defined lobules and poorly delineated cortex and medulla.

weight for the alcohol, control, and ACTH groups, respectively. No significant differences were apparent in these weights.

The histologic appearance of the thymuses from alcohol-treated and control animals is compared in Fig. 7. It can be seen that the thymus from the alcohol-treated animal is markedly atrophic. The lobules are not well delineated, and germinal centers are poorly formed. Centrally, there are large, indeterminate stem or reticulum cells, medium-sized lymphocytes, and only a few mature small lymphocytes. The thymus from the control animal is large, well lobulated, and shows a well-defined cortex and medulla containing prominent follicles with many perifollicular mature lymphocytes. The thymuses from ACTH animals did not appear different histologically from those of control animals.

Liver sections obtained from rats receiving the alcohol-formula diet showed significant fatty infiltration.

DISCUSSION

The results obtained in this study indicate that chronic administration of alcohol to rats results in abnormalities of both humoral and cellular immunity. In the alcohol-treated animals there was a delay in antibody production to both typhoid H and *B. abortus* antigens following primary immunization, although the peak titers were not significantly different from those of control animals. The secondary response to typhoid H antigen was not affected. In addition, chronic alcohol administration caused a depression of the cutaneous delayed-hypersensitivity response to a potent skin sensitizer, 2, 4-dinitrofluorobenzene.

The mechanism by which alcohol suppresses the immunologic system of the rat was not clarified by this study and remains poorly understood. It has been reported that in human beings with nutritional cirrhosis and in animals with experimentally induced cirrhosis, antibody formation is actually increased.^{13, 14} However, in these investigations, antibody studies were carried out at a time when the subjects were not ingesting consistently large amounts of alcohol.¹⁴ It should also be noted that cirrhosis was present in the subjects of the latter studies. In the present study, fatty infiltration of the liver was induced by the chronic administration of alcohol, but there was no evidence of cirrhosis. This type of model system allows for the separation of immune alterations due to chronic alcohol ingestion per se from changes due to the presence of cirrhosis.

The data obtained in this study suggest that suppression of reticuloendothelial function occurs with the chronic administration of alcohol. The delay in antibody production following primary immunization without suppression of the total antibody response may have been a manifestation of such an alteration in reticuloendothelial function. In this connection it has been demonstrated that acute administration of alcohol results in a significant depression of the clearance of intravenously administered aggregated albumin in rats.¹⁶ Guarneri and Laurenzi¹⁰ recently reported that mobilization of alveolar macrophages was impaired in alcohol-treated mice. This is of particular interest since it has been shown that the macrophage plays an important role in the initial processing of an antigen for maximal stimulation of the immunologically competent cell.¹⁷ Decreased macrophage function might therefore result in decreased antibody production. The decreased expression of cutaneous delayed hypersensitivity noted in the alcohol-treated rats may also be the result of altered reticuloendothelial function. If macrophage uptake of the applied antigen is delayed by alcohol treatment, full expression of reactivity may not be evident within 24 to 72 hours.

Neonatal thymectomy in many animal species, including the rat, has been shown to result in impaired development of the lymphoid system and consequently in defective development of delayed hypersensitivity.¹⁸ Thymectomy in adult animals, however, may not result in immediate impairment of immunologic reactivity, but immune defects may become evident later in life.¹⁸ In the present study chronic alcohol ingestion resulted in thymic atrophy, and this might account for the depressed cutaneous delayed hypersensitivity. The absence of total depression of delayed hypersensitivity may be due to the fact that the rats used in this study were young adults rather than neonatal animals and were not followed for a sufficient length of time. Santisteban¹⁹ has demonstrated that large doses of alcohol administered by subcutaneous injection to mice result in involution of the thymus. He has postulated that thymic involution was the result of increased secretion of adrenal corticosteroids. In the present study, however, it was demonstrated that prolonged administration of ACTH did not result in significant thymic involution. It could be argued that the lack of such involution may have been due to too small doses of ACTH given over an insufficient period of time. However, the adrenal weights of the

alcohol-treated animals were actually less than those found in the control animals. If the thymic atrophy found in the alcohol-treated animals was due to adrenal hyperactivity, one would have expected to find increased adrenal weights.

Although many factors have been postulated to account for the increased susceptibility of alcoholic human patients to infection, specific alterations in immune mechanisms have not been clearly defined. Data obtained in the present study indicate that, in rats, chronic alcohol ingestion has deleterious effects on both humoral and cellular immunity. Further studies are needed to determine whether similar effects of alcohol on the reticuloendothelial system or on thymic function occur in human alcoholic patients.

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