carotid circulation depends largely upon the nervous connections between the head and the trunk. On the other hand Cort (7), working with cats, has adduced evidence that there is a hormone released from the hypothalamus that is directly natriuretic. The present experiments provide no answer to the important question of exactly how the central nervous system may exert its influence on natriuresis.

On the basis of these experiments we cannot speculate on the details of the renal response that are manifest as saluresis. We did not measure glomerular filtration rate or renal plasma flow. Brooks and Pickford (1) found that these variables did not change after intracarotid oxytocin. We (2) have not seen consistent changes after intravenous oxytocin. Small increases in filtration rate, however, could account for the additional sodium excreted and still escape detection by clearance measurements.

Summary. Doses of oxytocin that are ineffective intravenously produce natriuresis when injected into the carotid arteries or the third cerebral ventricles of conscious dogs. These observations appear consistent with the hypothesis that oxytocin exerts its natriuretic effect by acting upon some intracranial structure rather than directly upon the kidneys.

The authors wish to express their appreciation for the kindness of Dr. S. C. Wang in demonstrating the technique for preparing carotid loops and to Dr. H. J. Bartelstone and Dr. H. C. Kwaan for surgical assistance in the implantation of ventricular cannulae.

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Inhibition of the Chronic Ethanol-Induced Fatty Liver by Antioxidant Administration* (32670)

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A new concept of the mechanism of acute ethanol-induced hepatic injury was proposed following the demonstration that antioxidant administration either prior to, or simultaneous with ethanol, effectively inhibited hepatic triglyceride accumulation and ethanol-corn oil induced hypertriglyceridemia (1–5). As a result of these observations and the known role of antioxidants as inhibitors of peroxidative lipid deterioration, an hypothesis was advanced that lipid antioxidants exerted their protective effects by preventing an ethanol-induced free radical chain reaction which would lead to the formation of lipid peroxides, hydroperoxides, and/or other complexes (1–7). More recent studies (6,7) have demonstrated increased peroxidation of hepatic lipids following *in vivo* or *in vitro* administration of ethanol. The enhanced lipid peroxidation *in vivo* occurred prior to the accumulation of triglycerides (6,7), but was not observed with brain homogenates which do not metabolize ethanol, denoting that the lipoperoxidation process may be related to ethanol metabolism, rather than ethanol, per se (7).

Porta and Hartroft (8) have confirmed

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the inhibitory effect of antioxidants on the acute ethanol-induced hepatic triglyceride accumulation. They also reported that the swelling and fusion of hepatic mitochondria, as well as mitochondria degeneration and dissolution noted after ethanol administration were effectively inhibited in antioxidant-treated rats. Other ethanol-induced hepatic cell ultrastructural changes were also modified, denoting the possible importance of lipid antioxidants in the stabilization and preservation of lipoprotein membranes. However, in marked contrast to the observations that antioxidants prevented the development of the acute ethanol-induced fatty liver (1–8), Porta *et al.* (9,10) and Lieber and De Carli (11) failed to inhibit the fatty liver induced by chronic ethanol ingestion by incorporation of a variety of antioxidants in the diet.

In view of the observation that antioxidants, such as vitamin E, are poorly absorbed from the gastrointestinal tract (12), the present studies were undertaken to evaluate the influence of the antioxidant, *N, N'*-diphenyl-*p*-phenylenediamine (DPPD) administered parenterally, on the hepatic triglyceride alterations induced by prolonged alcohol intake. Furthermore, since Lieber *et al.* (13) and Jones and Greene (14) have shown that the degree of steatosis induced by chronic ingestion of ethanol was dependent on the fat content of the diet, the influence of dietary lipid on the effectiveness of antioxidants to modify the chronic ethanol-induced fatty liver was also studied.

Methods. Female rats of the Sprague-Dawley strain (Dublin Laboratory Animals, Dublin, Va.) were maintained for a period of 2 weeks on Wayne Lab-Blox diet (Allied Mills, Inc., Chicago, Ill.) before beginning the experimental dietary period.

During the initial period, the antioxidant, DPPD, was administered intraperitoneally (60 mg/100 gm) as a suspension in corn-oil at 7 and 2 days prior to the commencement of the experimental feeding period. The antioxidant was also administered on the 12th day of experimental feeding. Control animals received an equivalent volume of corn oil intraperitoneally.

The experimental diets employed were derived from a readily available formula diet.[2] The first diet employed was the basic diet to which either sucrose or ethanol were added as 35% of the calories; this diet contained fat at 13% of the calories and was designated the "low" fat diet. The second diet was prepared by homogenization of 26.7 gm of corn oil per liter of the basic diet. Sucrose or ethanol were then added to provide 35% of the total calories; the fat content was calculated to be 23% of the calories. This diet was designated as the "high" fat diet. The volume and caloric composition of the respective sucrose and ethanol-containing diets were comparable, as were their vitamin and nutrient contents (Table I).

TABLE I. Composition of Experimental Formula Diets.

Ingredients	Units	"Low" fat diet	"High" fat diet
Caloric composition			
Protein	% of total calories	20.2	16.2
Fat	% of total calories	13.0	23.4
Carbohydrate	% of total calories	31.8	25.4
Ethanol or sucrose	% of total calories	35.0	35.0
Vitamin composition			
Vitamin A	USP Units/liter	4871	4759
Vitamin D	USP Units/liter	390	381
Vitamin C	USP Units/liter	97.4	95.2
Thiamine	mg/liter	1.9	1.9
Riboflavin	mg/liter	2.9	2.9
Niacinamide	mg/liter	14.6	14.3
Vitamin E	IU/liter	9.7	9.5
Pyridoxine	mg/liter	1.9	1.9
Vitamin B_{12}	μg/liter	1.9	1.9
Calcium pantothenate	mg/liter	9.7	9.5
Caloric value	cal/ml	1.3	1.7

Throughout the dietary period, the experimental animals were allowed to feed *ad libitum*. Diets were prepared three to four times weekly with refrigeration of excess. Fresh diet was supplied in drinking tubes every 24 hours to prevent evaporative loss of

[2] Chocolate Metrecal: Mead Johnson & Co., Evansville, Indiana.

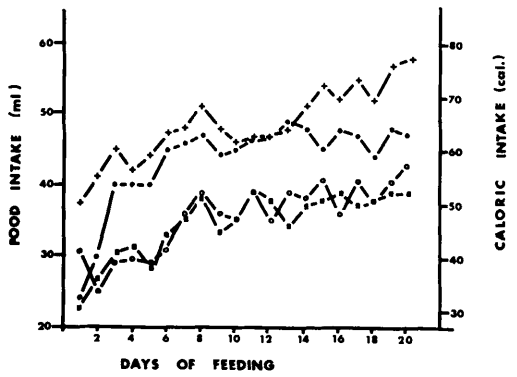

FIG. 1. Daily food and caloric intake of the liquid "low" fat diet in sucrose (+), sucrose + DPPD (●), ethanol (■), and ethanol + DPPD (○) treated rats. Each point is a mean of 9 animals per group.

ethanol. The volume of diet consumed and body weights were determined each day.

At the end of the 3-week dietary period, liver and plasma triglyceride concentrations were determined in duplicate on chloroform–methanol extracts (15) by the method of Van Handel and Zilversmit (16). Statistical analysis of the data was performed at the 95% confidence level by the *t* test for difference between means.

Results. Daily food consumption and caloric intake of the ethanol and sucrose-supplemented groups on the "low" fat diet is presented in Fig. 1. All animals manifested a progressive increase in food consumption during the initial 8-day period. Thereafter, food consumption in all groups stabilized with the exception of the corn-oil treated, sucrose-fed group which exhibited a secondary increase in food consumption which began on day 13. Throughout the experimental period, control and experimental animals on the alcohol diet consumed fewer calories than those on the sucrose diet.

Daily food consumption and caloric intake of animals on the "high" fat diet are presented in Fig. 2. In this experiment food consumption was essentially stable in all groups. In agreement with observations on the "low" fat diet, the animals on the ethanol supplemented "high" fat diet consumed significantly fewer calories per day than did the sucrose-fed animals. The caloric intake of comparable groups, i.e., sucrose or ethanol supplemented, on the "low" and "high" fat diets was, however, essentially identical.

On the "low" fat diet, the daily intake (gm/kg) of ethanol ranged from 9.6 to 15.9 in the control group and 9.6–16.1 in DPPD-treated ethanol group (Fig. 3). No significant difference existed in the mean intake per day of ethanol which was 13.3 ± 0.3 gm per kg per day in the ethanol-corn oil group and 13.9 ± 0.3 gm per kg per day in the ethanol–DPPD-treated group. Alcohol intake on the "high" fat diet ranged from 12.5 to 16.5 gm per kg per day in the control group and 11.4–17.4 gm per kg per day in the ethanol-treated

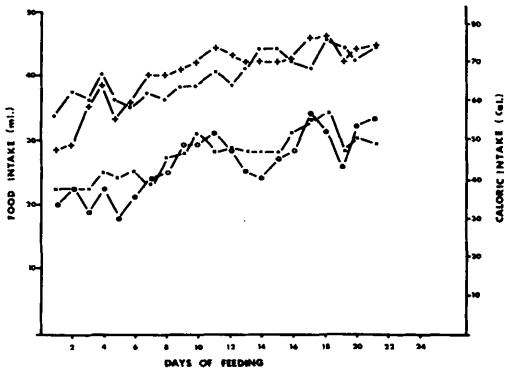

FIG. 2. Daily food and caloric intake of rats maintained on the "high" fat diet in sucrose (+), sucrose + DPPD (●), ethanol (■), and ethanol + DPPD (○) treated rats. Each point is derived from 6 animals per group.

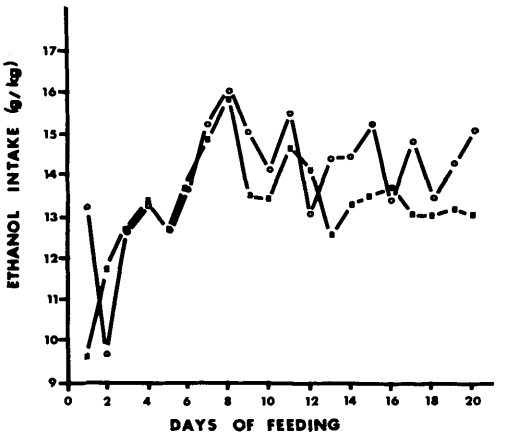

FIG. 3. The daily ingestion of ethanol in ethanol (○) and ethanol + DPPD-treated (■) rats maintained on the "low" fat diet. The DPPD was administered intraperitoneally in corn oil at −7, −2, and +12 days. The control ethanol group (○) received an equivalent volume of corn oil. Each point is derived from 9 animals per group.

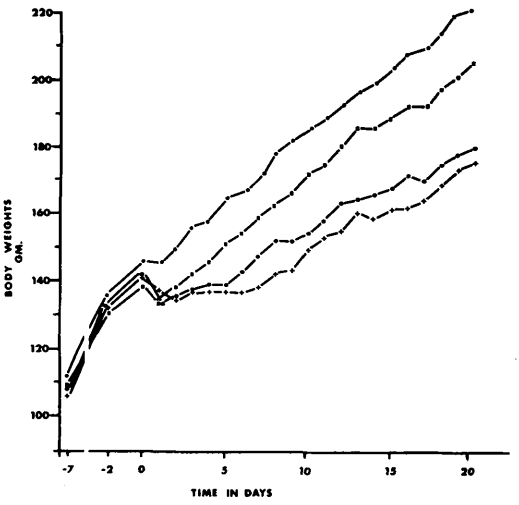

FIG. 4. Growth curves of rats treated with sucrose (●), sucrose + DPPD (■), ethanol (○), and ethanol + DPPD (+) and maintained on the "low" fat liquid diet. Each point represents a mean of 9 animals.

DPPD group. The average daily alcohol consumption in the "high" fat groups was 14.3 ± 0.2 and 14.4 ± 0.3 gm/kg in the control and DPPD-treated groups, respectively. These latter values were not significantly different than those found on the "low" fat diet.

The growth curves of rats consuming the "low" fat liquid diet are presented in Fig. 4. Although all of the rats began the experimental period with similar body weights, animals on the ethanol supplemented diets showed a significant inhibition in body weight gain in contrast to those animals maintained on the sucrose supplemented diet (Fig. 4, Table II). The administration of DPPD did not induce any significant alteration in body weight gain in the ethanol-treated group (Fig. 4, Table II). Comparison of Tables II and III indicates comparable weight gain occurred in animals maintained on both the "low" and "high" fat diets. In the latter animals, a significant decrease in weight gain in the ethanol-fed groups as compared to those fed the sucrose supplemented diet is again manifested. No difference occurred in body weight between control and DPPD-treated rats maintained on either the sucrose or ethanol supplemented diets permitting direct comparisons within these groups.

The control rats which received the ethanol supplemented "low" fat diet showed hepatic triglyceride concentrations which were elevated 115% over that observed in the isocaloric sucrose supplemented diet (Table II). In contrast, the rats maintained on the alcohol diet and treated with DPPD had hepatic triglyceride levels that were significantly reduced 46% from the ethanol group and which were equivalent to normal control values (6). The triglyceride concentrations in the DPPD group which received the ethanol supplemented diet were, however, significantly increased over the sucrose–DPPD–control group.

Hepatic triglyceride concentrations of rats maintained on the ethanol supplemented "high" fat diet were elevated 199% over similarly treated rats on the sucrose supplemented "high" fat diet. Treatment of

TABLE II. Influence of Antioxidant Administration on Liver Lipid Alterations Induced in Rats Maintained on an Ethanol-Supplemented "Low" Fat Formula Diet.[a]

Treatment		Body wt (gm)		Liver triglyceride (mg/gm)	Plasma triglyceride (mg/100 ml)
Dietary supplement	Intra-peritoneal	Initial	Final		
Sucrose	Corn oil	112 ± 3	223 ± 5	13.1 ± 0.6	40.7 ± 5.3
Sucrose	DPPD	110 ± 2	208 ± 3	9.5 ± 1.7	29.7 ± 3.9
Ethanol	Corn oil	109 ± 2	182 ± 7	28.0 ± 3.7	43.8 ± 5.3
Ethanol	DPPD	106 ± 3	176 ± 7	15.2 ± 1.1	64.4 ± 9.7

[a] Data expressed as the mean ± standard error of the mean are derived from 9 rats per group. The DPPD, suspended in corn oil, was administered intraperitoneally on days 7 and 2 prior to the feedings of the diet and on day 12 of the experiment. The control groups received corn oil alone.

TABLE III. Influence of Antioxidant Administration on Liver Lipid Alterations Induced in Rats Maintained on an Ethanol-Supplemented "High" Fat Formula Diet.[a]

Treatment		Body wt (gm)		Liver triglyceride (mg/gm)	Plasma triglyceride (mg/100 ml)
Dietary supplement	Intraperitoneal	Initial	Final		
Sucrose	Corn oil	109 ± 3	222 ± 4	17.8 ± 2.0	46.9 ± 13.6
Sucrose	DPPD	115 ± 5	217 ± 4	12.6 ± 1.6	63.5 ± 19.2
Ethanol	Corn oil	116 ± 4	179 ± 8	53.2 ± 8.8	62.5 ± 15.1
Ethanol	DPPD	115 ± 4	166 ± 8	28.3 ± 4.2	68.8 ± 11.1

[a] Data expressed as mean ± standard error of the mean are derived from 6 animals per group. The DPPD, suspended in corn oil, was administered intraperitoneally on days 7 and 2 prior to the feeding of the liquid diet and also administered on day 12 of the experiment. Control rats received corn oil intraperitoneally.

ethanol-fed animals with DPPD resulted in a significant 47% reduction in hepatic triglyceride. The hepatic triglyceride concentration in the ethanol–DPPD–treated group was, however, significantly increased over the sucrose-treated groups. The administration of DPPD to rats on the "high" fat sucrose diet resulted in a significant 29% reduction in hepatic triglycerides. Since food consumption in the control and DPPD-treated groups were the same, the decreased hepatic triglyceride concentration can be attributed to a direct effect of DPPD.

Plasma triglyceride concentrations in the sucrose supplemented group were similar to the comparably treated ethanol supplemented group (Table II). However, the rats maintained on the ethanol supplemented "low" fat diet and treated with DPPD had a plasma triglyceride concentration 58% greater than that of the sucrose-fed group treated with corn oil. On the other hand, DPPD treatment of the sucrose-fed rats led to a significant 14% decrease in plasma triglyceride concentration when compared to similarly fed corn oil treated rats. In contrast to these observations plasma triglyceride concentrations on the "high" fat supplemented diets were not significantly altered in the various groups (Table III).

Discussion. The present findings confirm previous observations that the chronic administration of ethanol in the amount of 36% of total calories results in a significant increase in liver total lipids and triglycerides (9–11, 13, 17). Hepatic triglyceride levels in the animals fed the "high" fat ethanol diet were significantly greater than that observed on the "low" fat ethanol diet under conditions of comparable caloric intake. This finding confirms the observations of Lieber *et al.* (11, 13), and Jones and Greene (14) that the degree of steatosis induced by prolonged ingestion of alcohol is dependent upon the fat content of the diet. Although Jones and Greene (14) observed increased hepatic lipids in chronic alcohol studies only when the dietary fat content exceeded 20% of the total calories, the present findings and those of Lieber *et al.* (13) denote that hepatic fatty changes can be induced with diets containing less than 20% fat. Indeed, the latter investigators (13) employed a 2% fat diet and observed a 1.5-fold increase in total hepatic lipids in ethanol-treated rats.

Lieber and De Carli (11) and Porta *et al.* (9, 10) observed that rats chronically maintained on ethanol-containing diets supplemented with antioxidants developed fatty infiltration of the liver which was equivalent to that observed in animals maintained on the ethanol diet alone. Our experiments demonstrate that the lipid antioxidant DPPD, administered by the intraperitoneal route, resulted in the maintenance of essentially normal hepatic triglyceride levels in rats maintained on ethanol supplemented "low" fat diets. Steatosis was significantly reduced by the DPPD, but not prevented, when rats were maintained on the "high" fat ethanol supplemented diet.

It is possible that the protective effect of

antioxidants noted in the present investigations, in contrast to the negative findings previously reported (9–11) may be due to the limited absorption of orally administered antioxidants (12) and the resulting inability to attain adequate cellular antioxidant concentrations. In support of this concept the production of thiobarbituric acid reactive material by liver homogenates of rats which received antioxidants orally (9, 10) was 25- to 40-fold greater than the concentration of hepatic thiobarbituric acid reactive material measured when DPPD was administered parenterally (6). The concentration of hepatic thiobarbituric acid reactive material formed *in vitro* by the peroxidation of lipids is inversely related to the antioxidant capacity of the tissue (6).

Porta *et al.* (9, 10) reported that hepatic thiobarbituric acid reactive material was significantly increased by the chronic administration of ethanol. A similar enhancement was also observed following the administration of a single acute dose of ethanol (6), denoting an enhanced lipid peroxidative process in both acute and chronic alcoholism. While liver antioxidant levels were significantly increased when α-tocopherol acetate was added to the formula diets (9, 10), the degree of inhibition of lipid peroxidation was significantly less than that achieved following the intraperitoneal administration of DPPD (6), as the concentration of hepatic thiobarbituric acid material was 52.8 μg/gm in the ethanol–vitamin E supplemented groups in the former study (9, 10), in contrast to 1.3 μg/gm in the latter study (6). The degree of antioxidant inhibition of lipid peroxidation necessary to inhibit or prevent the ethanol-induced biochemical (6) and structural alterations (8–10) is yet to be established.

It is also possible that the protective effect of the antioxidants may have been negated by the high dietary lipid content which was 43% of the total calories in the previous studies (9–11). In support of this alternate interpretation is the finding that increasing the dietary fat content from 13 to 23% of the total calories in the present study reduced the effectiveness of DPPD to maintain normal hepatic triglyceride concentrations.

De Carli and Lieber (17) recently reported the use of a new inexpensive liquid formula diet for studies on chronic alcoholism in rats. The diet employed in the present study, besides being nutritionally adequate, has the advantage that it is also inexpensive, readily available, and requires a minimum of time to prepare. This formula diet, which maintained normal hepatic triglyceride concentrations, and continued growth of the animal may be of value in future studies on chronic alcoholism.

The present data indicate that the lipid antioxidant, DPPD, which protects against the fatty liver induced by a large single dose of ethanol, is also effective in reducing steatosis resulting from prolonged ethanol ingestion. Furthermore, it is apparent that the fat content of the diet is an important factor not only in the production (13, 14), but also in the antioxidant modification of ethanol-induced liver injury. Although certain differences apparently exist in the acute and chronic ethanol models, such as the response to lipotropic factors (10), the present data of the ability of antioxidants to prevent or inhibit the fatty liver induced by prolonged ethanol ingestion suggests that the basic mechanism of enhanced lipid peroxidation, as initially proposed for the acute ethanol induced steatosis, may also be a factor in inducing the hepatic lesion in chronic alcoholism. These studies further accent the possible role of lipid antioxidants in the modification of hepatic injury.

Summary. Female rats were maintained for 21 days on nutritionally adequate liquid diets containing either "low" or high" fat content and supplemented with ethanol or isocaloric sucrose as 35% of the calories. Liver triglyceride concentrations were significantly elevated in animals maintained on both diets supplemented with ethanol. However, a greater increment in triglyceride concentrations was manifested in the "high" fat ethanol group under conditions of similar caloric intake. The intraperitoneal administration of the lipid antioxidant, *N,N'*-diphenyl-p-phenylenediamine (DPPD), prior to, and during the dietary period significantly inhibited the ethanol-induced increment in hepatic triglyceride concentration. The inhibition of the chronic ethanol-induced steatosis, in con-

juction with our previous studies of antioxidant modification of the acute ethanol-induced hepatic lesion, further accents the possible role of lipid peroxidation as a determinant in cell injury and the role of antioxidants as liver protective agents.

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Observations on the Inhibitory Influence of Plethora on Erythropoietic Action of Androgens in Hypophysectomized Rats* (32671)

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The present studies were prompted by two observations made in this laboratory: (a) that the erythropoietic response to norethandrolone, as measured by ^{59}Fe uptake and reticulocyte counts, was abolished by blood transfusions in hypophysectomized rats, and (b) whereas the hypophysectomized rat, 2 weeks after surgery, responds readily to norethandrolone, no response to equal doses of testosterone propionate was obtained (1). Further elucidation of the relationship between the plethoric state and androgen activity seems important to an ultimate understanding of the mechanisms by which androgens stimulate erythropoiesis.

The literature concerning the action of androgens on erythropoiesis has become extensive and many suggestions have been made on the mechanism of their stimulatory effect. In general, the following points of view are still in consideration: (a) androgens act by stimulating erythropoietin production (2–4) or they alter the sensitivity of the kidney in such a way that there is increased ESF production (3); (b) androgens act in some way other than by stimulating ESF production, such as an interaction in some manner with ESF (1,5) or with the stem cell so that stimulation occurs without requiring increased amounts of ESF (6); or (c) that androgens interfere or interact in some way with an inhibitor substance (7).

The use of the polycythemic or plethoric animal has been intimately involved in all the studies from which these various ideas have been formulated, and the plethora has influenced the response to androgens in a variety of ways. Gurney *et al.* (8–11) found, in the mouse, that various degrees of plethora would reduce, but not eliminate, the response

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