Differences in activation of complement-derived chemotactic factors (Di Perri & Auteri, 1974) may be part of the explanation for the differences seen in the inhibition of leucocyte migration. The inhibitory action of aspirin (possibly on C1 esterase) may be by-passed when carrageenan activates complement via the alternate pathway in the pleural model whereas that of indomethacin may not be so by-passed.

The authors thank Dr E. Khedouri (Biochemistry Division, North East London Polytechnic) for the supply of octanoyl salicylic acid.

July 19, 1978

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Alterations in the vascular compartment with acute ethanol treatment

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While investigating *in vivo* effects of ethanol on rat plasma, we became concerned about possible changes in the vascular compartment of treated animals. We therefore investigated the effects of three doses of ethanol on extravasation of large molecules from the vascular to the peritoneal compartment of rats. Ethanol, especially by intraperitoneal injection, effected considerable changes in the protein and Evans Blue content of the two fluid compartments, suggesting a shift in molecules from the blood to the peritoneal compartment, but only partially as a result of the local irritant or osmotic properties of ethanol.

Male Sprague Dawley rats (250–300 g) (Holtzman) were housed singly for one week before use in a room maintained on a 12:12 light/dark cycle and at 21°. Evans Blue was injected intravenously via the tail vein (2·5 g kg^{-1}). Ethanol (10 or 20% solution) was injected intraperitoneally or given intragastrically. Control rats received the equivalent volumes of saline. In most experiments, animals were decapitated and blood was collected into heparinized tubes. Haematocrits were rapidly determined by means of an International microcapillary centrifuge and reader. Blood was centrifuged at 8000 *g* to separate the cellular elements, and plasma was immediately separated and frozen at −20°. In some experiments, animals were killed by cervical dislocator (Wausau, WI 54401) and the peritoneal fluid was examined.

Peritoneal fluid was collected according to Dolphin, Elliott & Jenner (1976). Two ml of heparinized saline was introduced through a small slit made through the peritoneal wall. The hole was kept closed while the body was rocked gently from side to side to mix the peritoneal contents. The abdominal wall opening was then enlarged and the peritoneal fluid removed.

Evans Blue in plasma and in the exudate was measured spectrophotometrically. Absorbance of exudates was read at 605 nm after dilution (2·5×) with water

* Correspondence.

and centrifugation at 2000 *g*. Total proteins in plasma (after 1:150 dilution) and peritoneal exudate were determined by the method of Bradford (1976). The exudate was purified to minimize interference by the dye since it could not be diluted like the plasma samples because of its low protein content. After precipitation with 6% TCA, proteins in the exudate were sedimented (5000 *g*, 10 min). The resulting pellet was washed twice with water. The final pellet was dissolved in 0·3 M NaOH to give a solution that was used directly for the protein assay.

Blood ethanol was assayed by gas chromatography by the method of Redmond & Cohen (1972). Student's *t*-test was used to assess statistical significance. Results are expressed as means ± s.e.m.

In some experiments, animals were either adrenalectomized or splenectomized. Adrenalectomized animals were allowed one week to recover and splenectomized animals two days.

An effect of ethanol on the vascular compartment was indicated by its effects on the haematocrit (Table 1). The 1 g kg^{-1} dose had no effect, the 2 g kg^{-1} dose had a small effect, and the 4 g kg^{-1} dose resulted in a 15% increase in the haematocrit. Exposure to ethanol for two weeks (ingestion of a liquid diet; Freund, 1969) did not alter the haematocrit compared with those of animals receiving a similar diet containing sucrose (44·70 ± 0·35 *vs* 43·45 ± 0·89; n = 7 in each group). Nor was the haematocrit altered during withdrawal from ethanol (18 h: 43·81 ± 0·45, n = 7; 4 days: 42·3 ± 0·55, n = 7).

Table 1. *Changes in haematocrit after ethanol treatment of rats.* Animals were injected with saline or ethanol (20% solution, i.p.). They were killed at 30 min for the 1 g kg^{-1} groups and at 1 h for the 2 and 4 g kg^{-1} groups.

Treatment	Haematocrit	% Increase
Saline	41·25 ± 0·55	
Ethanol 1 g kg^{-1}	43·20 ± 0·45	4·7
Saline	41·20 ± 0·36	
Ethanol 2 g kg^{-1}	44·20 ± 0·39*	7·3
Saline	40·75 ± 1·15	
Ethanol 4 g kg^{-1}	46·97 ± 1·00**	15·3

* $P<0·05$ or more.

The increase produced by ethanol in the haematocrit could be the result of its action on the cellular or the fluid components of blood. To examine the second possibility, we determined the effect of a 4 g kg^{-1} dose of ethanol on plasma volume. The method of Wang (1959) was used: Evans Blue was injected intravenously. Blood samples (300 µl) were taken from a jugular cannula, implanted one week before the experiment, 10 min before and 10, 30, and 60 min after the dye injection. Ethanol or saline was injected intraperitoneally 15 min before the dye injection. Ethanol produced a 20% decrease in plasma volume (3·09 ± 0·17 *vs* 2·44 ± 0·22 ml/100 g; n = 8 in each group; $P<0·05$). This indicated that the main effect of ethanol is to induce fluid loss from the vascular compartment.

Our next experiment was intended to determine whether fluid was being lost into the peritoneal cavity. When Evans Blue was injected intravenously, even 1 g kg^{-1} ethanol increased slightly the appearance of dye in the peritoneal exudate (Table 2). This effect was dose-dependent. Part of the observed increase of dye and proteins with the 4 g kg^{-1} dose is due to the use of a 20% solution of ethanol *vs* the 10% used for the 1 and 2 g kg^{-1} injections. This was done to avoid excessively large injection volumes. The increase in peritoneal dye was 96·8 and 147·6% with the 2 and 4 g kg^{-1} doses of ethanol, respectively, and these doses also induced a rise in the protein content of the peritoneal exudate (Table 2), 68·7 and 100·9%, respectively. However, it was also apparent that just injecting a larger volume of saline intraperitoneally, such as the control for the 4 g kg^{-1} dose of ethanol, resulted in some migration of dye and proteins from the vascular compartment to the peritoneal fluid.

In this experiment, we also determined changes in proteins and dye in plasma (Table 2). There was no change in Evans Blue in plasma after 1 or 2 g kg^{-1} of ethanol, but the 4 g kg^{-1} dose decreased the dye content by 14·8% and elevated serum proteins by 19%. The decline in dye is due to its extravascular migration; the reason for the increase in serum proteins is less clear, but most likely is due to the effect of ethanol on the liver.

Next we examined the effect of the concentration of the ethanol solution injected (Table 3). A 1 g kg^{-1} dose was injected intraperitoneally as either a 10 or a 20% solution. The animals were killed 30 min later. The 20% solution elevated dye concentration in the peritoneal exudate by 41% but the same dose injected as a 10% solution had no significant effect. Protein content

Table 2. *Effect of ethanol treatment on concentration of Evans Blue and total proteins in peritoneal fluid and serum.*

	Serum		Peritoneal fluid	
Treatment	Evans Blue OD at 604 nm	Proteins mg ml^{-1}	Evans Blue OD at 604 nm	Proteins µg ml^{-1}
Saline	0·268 ±0·006	52·77 ±0·45	0·035 ±0·001	35·99 ±2·97
Ethanol 1 g kg^{-1}	0·287 ±0·009	58·32 ±2·02	0·048 ±0·005	40·58 ±2·95
Saline	0·238 ±0·001	57·82 ±1·58	0·064 ±0·004	37·30 ±3·01
Ethanol 2 g kg^{-1}	0·222 ±0·006	68·24 ±1·36*	0·126 ±0·005*	62·92 ±3·41*
Saline	0·264 ±0·007	48·53 ±0·65	0·124 ±0·020	42·73 ±7·21
Ethanol 4 g kg^{-1}	0·225 ±0·010*	58·15 ±1·58*	0·307 ±0·010*	85·84 ±17·00*

* $P<0·05$ or greater.

Table 3. *Effect of the concentration of the ethanol solution on the extravasation of Evans Blue and proteins.*

Peritoneal fluid		Ethanol solution 10%	20%
Dye (604 nm)	Saline	0·053 ± 0·005	0·049 ± 0·005
	Ethanol	0·062 ± 0·008	0·069 ± 0·003*
Proteins (μg ml^{-1})	Saline	39·65 ± 1·51	35·95 ± 1·98
	Ethanol	41·55 ± 1·99	40·80 ± 2·15*

* $P<0·05$.

of the exudate was elevated 13·5% by the 20% solution but not affected by the 10% solution. Values for the saline-injected animals did not differ in either the protein or dye content in the peritoneal fluid.

It is possible that the extravasation of dye and proteins occurring with intraperitoneal injections of ethanol was a side effect produced by the locally irritating properties of ethanol or was due to the osmotic effects of ethanol or saline within the peritoneal cavity. Therefore, we examined the effect of ethanol administered by a different route. A 4 g kg^{-1} dose of ethanol (20% solution) was given intraperitoneally or by gastric intubation, and animals were killed 2 h later. Ethanol by intubation still produced a 101% increase in dye and a 66% increase in proteins in the exudate (Table 4). Therefore, the extravasation of dye and proteins appears to occur irrespective of the mode of administration of ethanol, although the changes are less after intragastric administration.

Table 4. *Comparison of intraperitoneal and oral administration of ethanol on Evans Blue and protein extravasation.*

Peritoneal fluid		Ethanol treatment (4 g kg^{-1}) i.p.	Oral
Dye (604 nm, OD)	Saline	0·100 ± 0·008	0·099 ± 0·005
	Ethanol	0·266 ± 0·015*	0·199 ± 0·008*
Proteins (μg ml^{-1})	Saline	41·03 ± 2·35	41·75 ± 3·55
	Proteins	77·99 ± 2·69	69·25 ± 2·50*

* $P<0·05$.

The final experiment examined a possible mechanism for the extravasation of fluids and solutes as produced by ethanol. Sham-operated or adrenalectomized rats were given 4·0 or 3·7 g kg^{-1} of ethanol intraperitoneally (these doses has previously been determined to give equivalent concentrations of ethanol in blood) and killed 1 h later. Ethanol produced similar increases in the haematocrit (15·6 ± 0·8% *vs* 16·1 ± 0·4%; n = 7) and in the extravasation of proteins (108·8 ± 1·7% *vs* 101·4 ± 3·0%; n = 7) from blood in sham and operated rats, respectively. Thus, the effect of ethanol is not mediated by adrenocortical steroids nor by adrenal catecholamines.

An increase in the haematocrit with very high doses of ethanol has previously been reported in dogs by Klingman, Haag & Bane (1958) and Klingman, Bane & Haag (1959) who also noted that neither adrenalectomy nor splenectomy blocked the effect of ethanol on on the haematocrit.

In contrast to the short-term effect presented here, ethanol has been shown to have opposite long-term effects. Beard & others (1965) and Sargent, Simpson & Beard (1975) have found that in dogs ethanol (3 g kg^{-1}) produces an expansion of plasma volume and of extracellular fluid volume, as well as an increase in the total body water 18 h after treatment. Their studies confirmed similar findings obtained earlier in man and dogs (Nicholson & Taylor, 1940). These studies and the results presented here indicate that changes in blood volume should be considered, especially when examining the effects of ethanol on blood chemistry.

This work was supported by U.S. Public Health Service grants AA 00296 and AA 00014. We acknowledge the excellent technical assistance provided by Mrs Olga Irizarry.

May 25, 1978

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