Mechanism of Increased Hepatic Uptake of Unesterified Fatty Acid from Serum of Ethanol-Treated Rats

By MARC A. ABRAMS and CECIL COOPER

Department of Biochemistry, Case Western Reserve University, Cleveland, OH 44106, U.S.A.

(Received 27 August 1975)

Studies were made on the mechanism by which livers of ethanol-treated rats take up an increased fraction of the total flux of unesterified fatty acid in serum. It was found that ethanol (0.7 g/kg) causes a twofold rise in the serum content of liver, and that this serum is in rapid equilibrium with the general circulation. The fractional hepatic uptake from serum of a group of compounds with varying uptake mechanisms and metabolic fates was studied in control and ethanol-treated animals. All the compounds tested, including unesterified fatty acid, showed an enhanced uptake when ethanol was given. For one of the compounds, carbon tetrachloride, a dose/response relationship was established between the amount administered, the amount taken up by liver, and the amount metabolized. These findings were interpreted to mean that this dose of ethanol causes the liver to receive an increased flow of blood, and as a result all compounds present and capable of being taken up by liver are taken up at an increased rate. Hepatic blood flow was measured by a technique that monitors the rate of clearance of a colloidal lipid emulsion. It was found that ethanol increased hepatic blood flow by about 60%. This effect of ethanol on hepatic blood flow provides an explanation for the fatty liver and the synergistic effect between an acute dose of ethanol and carbon tetrachloride. A hypothesis to explain why a moderate dose of ethanol causes triglyceride to accumulate in liver is presented.

In the preceding paper (Abrams & Cooper, 1976) the intraperitoneal injection of ethanol (0.7 or 2.1 g/kg) into rats was shown to cause a linear increase in liver triglyceride for at least 2 h. It was also shown that with a low dose of ethanol (0.7 g/kg) all the excess triglyceride synthesized may be accounted for quantitatively by increased hepatic uptake of unesterified fatty acid from serum.

The uptake of unesterified fatty acid by liver or Ascites cells has been shown to be directly proportional to the concentration to which they are exposed (Armstrong *et al.*, 1960; Fine & Williams, 1960; McElroy *et al.*, 1960; Spector, 1968). An elevated concentration in serum could conceivably explain the excess fatty acids entering liver, but this concentration was measured and found to be unaffected by ethanol under our experimental conditions [see the preceding paper (Abrams & Cooper, 1976)]. If the total movement of unesterified fatty acid through the serum compartment (flux) is elevated by increased lipolysis in adipose tissue, this could also result in enhanced hepatic uptake with no change of serum concentration. We found that ethanol had no effect on total flux in our system (Abrams & Cooper, 1976) thereby leaving only one tenable concept, namely an increased hepatic clearance of unesterified fatty acid from serum. The

fact that there is no change in flux and serum concentration of fatty acid means an increased hepatic clearance could only occur if there were an accompanying decrease in extrahepatic clearance. Such a shift in whole-body clearance of unesterified fatty acid from serum has been observed [see Table 5 in Abrams & Cooper (1976)].

Hepatic clearance is the product of the volume of blood flowing through liver per min (hepatic blood flow) and the extraction efficiency of this organ for the compound being cleared. An enhanced flow to liver may arise via the opening of additional hepatic sinusoids, an increase in hepatic perfusion pressure, or a combination of both. An increase in hepatic blood flow could result in an increase in the amount of liver mass being perfused effectively by the hepatic vascular network. Enhanced extraction efficiency could increase the fraction of unesterified fatty acids removed from serum during a single passage of blood through liver. This could come about: (1) from a specific effect on uptake of unesterified fatty acids from serum; (2) by opening additional sinusoids (increasing absorptive surface) without altering hepatic blood flow; or (3) by other less well defined effects such as change in temperature (Brauer, 1963). The main distinction between the first two possibilities for increasing extraction efficiency

is that the first affects only fatty acids, whereas the second is a more general phenomenon that should affect any compound normally taken up by liver.

The purpose of the work described below is to discover whether an acute dose of ethanol can affect hepatic blood flow.

Experimental

Experiments with ^{125}I -labelled albumin

Albumin and all other compounds injected intravenously were adjusted to a final volume of 0.5 ml with serum freshly prepared from rats starved for 48 h. Animals were injected in a tail vein with 0.5–1.0 μCi of ^{125}I -labelled human serum albumin (Squibb Co., New York, NY, U.S.A.), then 60 min later either ethanol (0.7 or 2.1 g/kg) or an equal volume of water was injected intraperitoneally. At specified times after ethanol injection the rats were decapitated and blood collected from the neck directly into a centrifuge tube for 5 s, allowed to clot for 45–60 min and centrifuged for 20 min at 500g, all at room temperature (22°C). Livers from decapitated rats were rinsed twice in cold 0.15M-NaCl, blotted dry, divided as described in the preceding paper (Abrams & Cooper, 1976) and the small lobe (weighing about 1 g) was placed directly in a tube for determination of radioactivity. The livers were perfused *in situ* by decapitating the rat, opening the peritoneal cavity, inserting a cannula in the hepatic vein, cutting the portal vein and hepatic artery and quickly forcing through 20 ml of 0.15M-NaCl. This entire operation from the time of decapitation to completion of the perfusion took about 5 min. The livers were then excised and handled as above.

To determine whether the serum in liver was in full equilibrium with the general circulation, experiments were carried out as described above, except that the ethanol (0.7 g/kg) was administered 30 min before ^{125}I -labelled albumin. At specified short intervals thereafter the animals were decapitated, and serum and livers prepared for gamma-radiation counting as described above.

Experiments with carbon tetrachloride

Animals were given ethanol (0.7 g/kg) or an equal volume of water, then 60 min later 0.1–0.2 μCi of [^{14}C]carbon tetrachloride (New England Nuclear Corp., Boston, MA, U.S.A.) was injected intraperitoneally with a Hamilton syringe in doses of 0.5–7.5 μl of carbon tetrachloride/100 g body weight. The [^{14}C]carbon tetrachloride injected was not diluted with unlabelled carbon tetrachloride, or dissolved in any carrier such as mineral oil. We found that when [^{14}C]carbon tetrachloride dissolved in mineral

oil was injected intraperitoneally, the rate of appearance of ^{14}C in liver was related to the concentration of carbon tetrachloride in the oil as well as the volume of solution injected. This made interpretation of the data ambiguous and prompted us to inject the [^{14}C]carbon tetrachloride undiluted. A short time (2 min) after injection of isotope the rats were decapitated, blood collected, livers removed as described above and frozen. The frozen lobe of liver (1 g) was homogenized in 20 ml of toluene/methanol (2:1, v/v). Duplicate 8 ml samples of the upper toluene layer were transferred to 20 ml scintillation-counting vials. To one vial 7 ml of toluene scintillant (Lowenstein, 1971) was added. Toluene in the second vial was evaporated at 50°C. The non-volatile lipid residue was redissolved in 15 ml of toluene scintillant and the radioactivity of both samples determined. The non-volatile radioactivity found in the vial that had been taken to dryness is believed to be covalently bound to lipid (Rao & Recknagel, 1968). This assumption is justified, since little if any unmetabolized carbon tetrachloride is converted into lipid-soluble, non-volatile products (Butler, 1961; Paul & Rubinstein, 1963).

Experiments with other compounds

Animals were injected with ethanol (0.7 g/kg) or an equivalent volume of water, then 30 min later injected in a tail vein with a pulse of one of the following compounds: 2–3 μCi of [$7\text{-}^{14}\text{C}$]benzoic acid; 0.24 μCi of L-[$1\text{-}^{14}\text{C}$]glutamic acid; 14–16 μCi of 3-O-[^3H]methylglucose (all from New England Nuclear Corp.); or 7 μCi of a colloid of sulphur labelled with technetium 99m (Squibb Co.). Then 2 min after injection with benzoate, glutamate, or 3-O-methylglucose, the animals were decapitated, and the blood collected from the neck. Serum to be used for liquid-scintillation counting was prepared as described above. The livers were removed, rinsed twice in cold 0.15M-NaCl, blotted dry, divided as previously described, weighed, and the same small lobe (about 1 g) was homogenized at room temperature in 30 ml of 0.15M-NaCl in a Sorvall Omnimixer for 3 min at low speed. The homogenate was adjusted to 39 ml with 0.15M-NaCl. A portion (1 ml) of 1M-NaOH was added to aid solubilization of tissue, and the clear homogenate used for liquid-scintillation counting.

The animals injected with colloidal sulphur were decapitated after either 1 or 2 min, and the livers and serum prepared and analysed for radioactivity exactly as in the experiments with labelled albumin. Because of the extremely rapid decay of this material (half-life approximately 6 h) a 0.1 ml sample was saved to serve as a standard for the total radioactivity injected, since data is expressed on the basis of percentage of injected dose.

Determination of hepatic blood flow

The technique of using the clearance of a colloid to determine hepatic blood flow has been employed frequently (Dobson & Jones, 1952; Benacerraff *et al.*, 1957; Biozzi *et al.*, 1958; Razzak & Wagner, 1961).

We decided to use a lipid-containing colloid. An anhydrous base similar to that described by Zilversmit *et al.* (1956) was prepared as follows. Corn oil, glycerol and soya phosphatidylcholine (Centrophil SM, Central Soya Co., Chicago, IL, U.S.A.) (10:10:1, v/v/w) were homogenized in a Sorvall Omnimixer for 10 min to a 'honey-like' consistency. This crude soya phosphatidylcholine preparation was necessary as purified material was insoluble in corn oil or glycerol. It was found that the soya phosphatidylcholine must first be dissolved in corn oil and the glycerol then added gradually while mixing, and finally 10 μ Ci of tri([1- 14 C]oleate) (Amersham-Searle, Arlington Heights, IL, U.S.A.) was added per ml of anhydrous base. A 10% (v/v) gelatinized emulsion of lipid, with particles ranging in size between 1–3 μ m (Saba *et al.*, 1966), was prepared by combining the above mixture with 0.1% purified pig skin gelatin (Fisher Chemical Co., Silver Springs, MD, U.S.A.) in 5% (w/v) dextrose and water. The dose was from 2–10 mg of triglyceride/100 g body wt. and was prepared by diluting an appropriate volume of emulsion to 0.5 ml with serum freshly prepared from rats starved 48 h. The resulting serum solution was injected intravenously into rats that had been administered either ethanol (0.7 g/kg) or an equal volume of water 30 min earlier. At specified times after injection of radioisotope the animals were decapitated, the blood collected, and serum prepared as described above. The lipids were extracted from the frozen lobe or the entire frozen spleen by homogenizing in 20 ml of redistilled chloroform/methanol (2:1, v/v) and 'washed' by the method of Folch *et al.* (1957). A 10 ml portion of the lower phase (chloroform) containing total lipids was transferred to a 20 ml scintillation vial and evaporated to dryness at 50°C before counting for radioactivity.

Liquid-scintillation counting

Samples of serum (0.2 ml) were made up to 1.0 ml with water, 3.0 ml of Aquasol (New England Nuclear Corp.) added, and the mixture shaken to form a gel. A portion (1 ml) of the alkaline homogenate from the experiments with radioactive glutamate, benzoate and 3-*O*-methylglucose was mixed with 3.0 ml of Aquasol, and the mixture shaken to form a gel. The dried extracts of chloroform from liver and spleen were dissolved in 4.0 ml of Bray's solution (Bray, 1960). All 14 C and 3 H radioactivity was counted in a Nuclear-Chicago mark I

liquid-scintillation counter. An external standard was used to determine the counting efficiency of each sample. Radioactivity is expressed as d.p.m. Counting efficiencies were 50–60% for samples of serum, 75–85% for carbon tetrachloride in toluene scintillant, 40% for homogenates containing benzoate and glutamate, 5–10% for 3 H (3-*O*-methylglucose) and 70–80% for extracts of liver and spleen. 125 I and technetium 99 m were counted with a Beckman gamma counter.

Results and Discussion

The focus of the work described below is on the mechanism by which ethanol enhances uptake of unesterified fatty acid from serum. Since the concentration and total flux of unesterified fatty acid in serum are unaffected (Abrams & Cooper, 1976), the most reasonable explanation is that ethanol effects hepatic clearance. This parameter may be altered by a change either in blood flow to liver, or in hepatic extraction efficiency, or by a change in both. The present experiments are designed to investigate whether ethanol can affect the uptake of unesterified fatty acid from serum by altering the flow of blood to liver.

Effect of ethanol on serum content of liver

To test the possibility that ethanol increases the fraction of total liver mass being effectively perfused we adapted a technique used by Felts (1965) and Belfrage (1966) to study the uptake of chylomicrons by liver. They found that 70–80% of the radioactive chylomicrons that seem to be removed from circulation by liver could be washed out by a brief perfusion. They concluded that the total radioactivity in liver represents chylomicrons trapped in sinusoids rather than uptake into hepatocytes. In the present experiments 125 I-labelled albumin was injected intravenously instead of labelled chylomicrons and the effect of ethanol on the amount of label trapped in the hepatic sinusoids is seen in Fig. 1. The amount of radioactivity in serum was measured simultaneously. Since albumin disappears from serum at an insignificant rate during these short experimental periods, it is assumed that any radioactivity in liver represents 'trapped' serum. There is an approximately twofold increase in retention of 125 I-labelled albumin in livers of animals receiving ethanol. The value we obtained for volume of serum in livers of control animals (0.1 ml/g of liver) can be converted into volume of blood trapped in liver by assuming that the hematocrit value obtained from peripheral blood (0.49) also applies to blood in liver. Our value for blood content of liver is in reasonable agreement with the value of 24 ml of blood/100 g of liver reported by Greenway & Stark (1971). The

Fig. 1. Pooling of ^{125}I -labelled albumin in liver

All animals were injected into a tail vein with ^{125}I -labelled serum albumin. At specified times after intraperitoneal injection of water (Δ) or ethanol in doses of 0.7 g/kg ($\square$) or 2.1 g/kg ($\bullet$), the animals were decapitated and livers and sera were analysed for radioactivity as described in the Experimental section. Each point represents results from one animal.

Fig. 2. Rapid equilibration of ^{125}I -labelled albumin

Animals were injected intraperitoneally with 0.7 g of ethanol/kg, then 30 min later ^{125}I -labelled serum albumin was injected into a tail vein. At specified times the animals were decapitated and the livers and sera analysed for radioactivity as described in the Experimental section. Each point represents results from one animal.

time-course, relative magnitude of the change, and lack of a relationship between dose of ethanol given and amount of serum found in liver correlates well with the effect of ethanol on hepatic uptake of unesterified fatty acid from serum (Abrams & Cooper, 1976). About 80% of the isotope can be washed out by rapid perfusion with 20 ml of 0.15 M-NaCl in control as well as

ethanol-treated rats. This agrees with the results of Felts (1965) and Belfrage (1966).

To determine if the increased amount of serum in liver after ethanol administration is in equilibrium with the general circulation, the sequence of the above experiment was reversed. The lower dose of ethanol (0.7 g/kg) was administered, and 30 min later, when the serum content was increased (Fig. 1), ^{125}I -labelled albumin was injected into a tail vein. At short intervals after injection of isotope the animals were decapitated and analysed as described above; the results are shown in Fig. 2. The extremely rapid rise in radioactivity in liver to the same extent as that seen in Fig. 1 indicates that this extra volume of serum in liver is in full equilibrium with the general circulation and does not represent entrapment in closed sinusoids.

The simplest explanation of the increase in hepatic serum content is that ethanol causes an increase in hepatic blood flow. Such an explanation could accommodate either a transitory or sustained change in hepatic blood flow.

Hepatic uptake of various compounds

A change in the number of sinusoids participating in the hepatic circulation would result in an increase in the amount of absorptive surface area available and should enhance uptake of any blood-borne substance normally taken up by liver, rather than being specific for the uptake of unesterified fatty acids. To test this idea, pulses of a variety of compounds known to be taken up by liver were injected intravenously to determine whether a small dose of ethanol (0.7 g/kg) enhances their uptake in a manner similar to the effect observed with unesterified fatty acid of serum. The compounds tested were: (1) 3-*O*-methylglucose, a non-metabolizable analogue of glucose; (2) benzoic acid, converted to hippuric acid in liver; (3) L-glutamate, a normal hepatic metabolite; and (4) colloidal sulphur labelled with technetium 99 m that is removed from circulation by Kupffer cells and used clinically to carry out 'liver scans'. Each compound showed an enhanced uptake when injected 15 min after administration of ethanol, and the degree of enhancement was similar to that observed with unesterified fatty acid (Fig. 3). Colloidal sulphur was found to be taken up at an elevated rate in ethanol-treated rats both at 1 and at 2 min after injection of isotope when very different amounts of the total dose injected had been cleared. This is in keeping with the ethanol-enhanced uptake of unesterified fatty acid from serum which is constant at all times after injection of isotope (Abrams & Cooper, 1976). The fact that all these compounds showed a similar pattern demonstrates that the effect we have observed is not specific for the transport of fatty acid into the hepatocyte.

Fig. 3. Effect of ethanol on hepatic uptake of various compounds

Animals were injected intraperitoneally with 0.7 g of ethanol/kg or an equivalent volume of water, then 15 min later one of the compounds was injected into a tail vein. At 2 min after injection of all compounds [except for the sulphur colloid which was injected at 2 min and at 1 min (right-hand pair)] the animals were decapitated and the livers and sera analysed for radioactivity as described in the Experimental section. Open bars represent values obtained from control rats (injected with water) and hatched bars from animals receiving ethanol. Horizontal bars denote the percentage of the injected dose that left the blood. All values have been corrected for serum content of liver.

Interactions between ethanol and carbon tetrachloride

Since ethanol and carbon tetrachloride have a known synergism (Traiger & Plaa, 1972; Hasamura *et al.*, 1974), we tested the effect of ethanol on the uptake and metabolism of radioactive carbon tetrachloride. The damage that carbon tetrachloride causes to liver results from its metabolism (Recknagel & Glende, 1973). If ethanol increases hepatic uptake of carbon tetrachloride and if enhanced uptake results in greater metabolism, then this action of ethanol could explain the synergism in the case of an acute dose of ethanol. The experimental parameter we used to monitor carbon tetrachloride metabolism is the amount of radioactive carbon originally injected as [^{14}C]carbon tetrachloride that is recovered bound covalently to lipid (Reynolds, 1967; Gordis, 1969; Recknagel & Glende, 1973). It is believed that carbon tetrachloride toxicity requires a homolytic cleavage of a carbon-chloride bond, resulting in the production of trichloromethyl free radicals in the vicinity of microsomal-membrane phospholipids with a high density of polyenoic

Fig. 4. Effect of ethanol on carbon tetrachloride that becomes bound to total liver lipid

Animals were injected intraperitoneally with water or 0.7 g of ethanol/kg, then 60 min later [^{14}C]carbon tetrachloride was injected intraperitoneally in the specified dose. At 2 min after administration of isotope the animals were decapitated and analysed for radioactivity as described in the Experimental section. Each point [controls (●), or ethanol-treated (○)] represents the mean $\pm$ S.E.M. For the dose of 1 μl of carbon tetrachloride/100 g body wt., 18 control and 15 ethanol-treated rats were used. Results for all other doses are based on five rats. Lipid-bound carbon tetrachloride is actually carbon tetrachloride (μg) that has 'become bound covalently' to an unsaturated fatty acid (Recknagel & Glende, 1973).

fatty acids. Some trichloromethyl free radicals could remove highly reactive methylene carbon and hydrogen atoms, generating organic free radicals involving fatty acids. These fatty acid free radicals are presumed to react with either molecular oxygen to form organic peroxides or another trichloromethyl free radical, resulting in some radioactivity being bound to lipid. The key feature of carbon tetrachloride toxicity is considered to be the lipid peroxidation and destruction of membrane integrity (Recknagel & Glende, 1973). The covalent binding of a trichloromethyl free radical to a fatty acid free radical on a membrane was shown not to be detrimental in itself (Glende *et al.*, 1975), but is probably a reflection of the extent to which fatty acid free radicals capable of interacting with oxygen have been generated. The latter type of interaction is presumed to lead to peroxidation of lipids and cellular damage.

With extremely low doses of carbon tetrachloride, a dose/response relationship was established between the amount of [^{14}C]carbon tetrachloride injected and the amount of radioactive carbon that becomes bound covalently to total liver lipid (Fig. 4). This effect was not seen in earlier studies by Ugazio *et al.* (1973) where with doses of either 25 or 500 μl of carbon tetrachloride/100 g body wt., the same amount of 'carbon tetrachloride', presumably in the form of a trichloromethyl group, in relation to total lipids was found in liver. The establishment of a dose/

response relationship raises the possibility that the synergism observed between carbon tetrachloride and acute administration of ethanol (Traiger & Plaa, 1972) may be explained by an enhanced uptake and metabolism of carbon tetrachloride similar to that observed with all other compounds tested (Fig. 3). Since only a small fraction of carbon tetrachloride administered actually reaches liver and then becomes bound to total liver lipid (as observed in our laboratory), it is possible that ethanol increases the hepatotoxicity of carbon tetrachloride by causing more to be taken up and metabolized. As seen in Fig. 4, ethanol does increase the amount of 'carbon tetrachloride' bound to lipid, making a dose of $1.0\mu\text{l}$ of carbon tetrachloride/100g body wt. respond like a dose of $1.9\mu\text{l}$ of carbon tetrachloride/100g body wt. in terms of metabolism within the liver. The total amount of carbon tetrachloride found in liver was also found to be dose-dependent from $0.5\text{--}7.5\mu\text{l}$ of carbon tetrachloride/100g body wt. This is the first time anyone has shown a direct relationship between the amount of carbon tetrachloride administered, the amount delivered to liver, and the amount converted into a lipid-bound form. Although the doses used in these studies are sublethal, they are within the range known to affect liver. A low dose ($25\mu\text{l}$) of carbon tetrachloride has been shown to protect a rat from an otherwise-lethal dose of $500\mu\text{l}$ of carbon tetrachloride/100g body wt. (Ugazio *et al.*, 1973). Also, fatty liver resulted from administration of doses as low as $1\mu\text{l}/100\text{g}$ body wt. (Recknagel & Goshal, 1966). Studies such as these

indicate that low doses may produce significant physiological effects, therefore the type of study presented here on the effect of ethanol on the disposition of carbon tetrachloride may prove useful for studying various ethanol/drug interactions.

Effect of ethanol on hepatic blood flow

It is possible that ethanol produces an increase in the amount of unesterified fatty acid taken from serum into liver by altering hepatic blood flow. Increased delivery of blood to liver may be the reason why excess serum is found in livers of animals administered a low dose of ethanol. Hepatic blood flow can be measured by direct or indirect methods. Direct methods are very difficult to apply with animals as small as rats and indirect techniques are customarily used. These involve measurement of the rate of clearance from blood of substances removed entirely or primarily by liver (Witte & Witte, 1974; Brauer, 1963). To make this estimate we used a colloidal lipid cleared primarily by Kupffer cells in liver.

Table 1 shows the values for t_4 obtained with several doses of colloid. Doses of 2 and 4mg of triglyceride/100g body wt. gave approximately the same result, whereas 10mg of triglyceride/100g body wt. was cleared at a lower rate. The simplest interpretation is that the highest dose exceeds the liver's capacity to maintain a maximum clearance rate whereas at subsaturating doses of colloid the clearance rate obeys first-order kinetics and is dependent only on the amount of blood perfusing

Table 1. *Effect of ethanol on hepatic blood flow*

The dose of colloid is expressed in mg of triglyceride/100g body wt. Ethanol (0.7g/kg) was administered 30min before colloid. n is the total number of animals used to determine t_4 . Equal groups of animals were killed at 8, 15, 26 and 40s after isotope injection, except for the Expt. no. 4 control group, where only the 15 and 40s measurements were made. The time that it takes for half the injected dose to be removed from serum (t_4) was determined by linear regression. Hepatic blood flow is defined as the blood volume (ml/100g body wt.) $\times K$ [fractional hepatic uptake (min^{-1})]. The second term, K , is equal to $0.693/t_4$ and was corrected for uptake by liver plus spleen only by using the data in Table 2, i.e. $0.693/t_4 \times \text{injected dose (\%)} \text{ taken up by liver plus spleen}$. The serum volume determined by isotope dilution of ^{125}I -labelled albumin as described in the preceding paper (Abrams & Cooper, 1976) was converted into blood volume by dividing by the hematocrit ($49 \pm 0.4\%$, $n = 32$). The radioactivity (d.p.m./ml of serum) obtained by extrapolating to zero time divided into the amount of radioactivity injected ($0.1\text{--}0.5\mu\text{Ci/rat}$) also gives an estimate of the serum volume. The value obtained in this manner was slightly higher (15%) than that obtained with the labelled albumin. The coefficient of error for the measurement of hepatic blood flow is approximately $\pm 7\%$.

Expt. no.	Colloid dose	Ethanol	n	t_4 (min)	K (min^{-1})	Hepatic blood flow (ml/min per 100g body wt.)
1	2	—	12	0.27	2.18	18.9
2	4	—	12	0.24	*	*
3	10	—	12	0.64	†	†
4	2	—	6	0.25	2.35	20.3
4	2	+	16	0.18	3.52	30.5
5	2	—	24	0.27	2.18	18.9
5	2	+	24	0.20	3.16	27.3

* Not calculated because recovery data (Table 2) is based on a different dose of colloid.

† Does not obey first-order kinetics.

Table 2. *Uptake of lipid colloid by tissues*

The six animals in each group received an intravenous colloidal dose of 2mg of triglycerides/100g body wt. Ethanol-treated rats were administered an intraperitoneal dose of 0.7g/kg and control animals an equivalent volume of water 30min before injection of colloid. All animals were decapitated 40s after the lipid emulsion was injected.

	Uptake (% of injected dose; mean $\pm$ S.E.M.)	
	Control	+Ethanol
Liver	67.1 $\pm$ 2.2	78.4 $\pm$ 1.2
Spleen	17.8 $\pm$ 0.6	12.9 $\pm$ 0.6
Lung	12.2 $\pm$ 1.0	6.6 $\pm$ 0.3
Blood	1.7 $\pm$ 0.1	1.0 $\pm$ 0.1
Recovery	98.8 $\pm$ 2.5	98.9 $\pm$ 1.4

the liver if the extraction efficiency is constant. If the extraction efficiency is known and if the clearance is only the result of uptake by liver, then hepatic blood flow may be calculated. However, several of these assumptions are not valid. Ideally, the substance should be cleared 100% on each pass through liver, in which case the rate of clearance would be directly related to the rate of hepatic blood flow. Although the extraction efficiency of this particular colloid has not been measured in rats (Saba *et al.*, 1966), for CrPO₄ colloids it has been shown to average 84% in dog, rabbit and mouse (Dobson & Jones, 1952) and for colloidal albumin it also averages 84% in rats (Benacerraf *et al.*, 1957). Therefore, our measurement will provide only a minimum estimate. Another assumption is that only liver is clearing the colloid; the data in Table 2, however, show this is not the case. Approx. 13–18% of the injected dose is in spleen and 7–12% is in lung. Since the rate of colloid clearance is not entirely the result of uptake by liver, the value of K (fractional hepatic uptake; shown in Table 1) was determined by multiplying $0.693/t_{1/2}$ by the fraction of total clearance attributable to liver plus spleen (Table 2) (Dobson & Jones, 1952). Hepatic blood flow measurements in rats have been reported by others: Dobson & Jones (1952) found that the half-time for clearance of CrPO₄ colloid from blood averaged 0.5min, which is reasonably close to our values (Table 1). We have assumed that differences in clearance rates of the lipid colloid (Table 1) are primarily a reflection of changes in blood flow rather than extraction efficiency for the following reasons. The recovery of colloid in liver plus spleen is high and not very different in both control and ethanol-treated animals (Table 2). A number of very different substances appear to be taken up more readily by liver after ethanol administration (Fig. 3). It is more probable that this

results from an increase in blood flow rather than comparable effects on extraction efficiency in each individual case.

This finding, that ethanol enhances hepatic blood flow, is of potential importance in understanding the known synergism between ethanol and a variety of drugs. Branch *et al.* (1973) reported that propranolol decreased the clearance of lidocaine by virtue of its effect on hepatic blood flow. The role of haemodynamics in drug clearance and toxicity has been reviewed (Wilkinson, 1975).

Our results must also be considered when interpreting those of Israel *et al.* (1975). These workers concluded that chronic ethanol administration induces a hypermetabolic state in liver that results in local decreased oxygen tension and liver damage. However, this interpretation is predicated on the assumption hepatic blood flow is not altered by ethanol. It remains to be determined whether hepatic blood flow is also increased in animals treated chronically with ethanol.

Our hypothesis explaining why a moderate dose of ethanol (0.7g/kg) causes triglyceride to accumulate in liver is as follows. The intestinal absorption of ethanol following an intraperitoneal injection either decreases intrahepatic resistance to blood flow or increases blood flow into the portal vein via the superior mesenteric vein. The latter could easily be accomplished, since 75–80% of the portal venous blood flow comes from the gastrointestinal tract (Grim, 1963) and would lead to an enhanced hepatic blood flow. Either a decrease in hepatic resistance or an increase in portal blood flow would result in an increase in the number of hepatic sinusoids perfused. An increased absorptive surface becomes available and there is in turn an increased amount of unesterified fatty acid absorbed from blood. The rate of synthesis of triglyceride appears to be determined by the availability of fatty acid and as a consequence formation of triglyceride goes up. Since there is no marked change in the rate at which hepatic triglyceride is released into blood as lipoprotein, fatty liver results.

The present work was supported by grants from the Northeast Ohio Chapter, American Heart Association, the Regional Council on Alcoholism of Cleveland, OH, U.S.A., the Department of Health of Ohio, and a training grant (GM-00035) from the National Institutes of Health. Some of this work forms part of a thesis presented by M. A. A. in partial fulfilment of the requirements for the Ph.D. degree at Case Western Reserve University, Cleveland.

References

- Abrams, M. A. & Cooper, C. (1976) *Biochem. J.* **156**, 33–46

- Armstrong, D. T., Steele, R., Altszuler, N., Dunn, A., Bishop, J. S. & DeBodo, R. C. (1960) *Am. J. Physiol.* **201**, 9-15
- Belfrage, P. (1966) *Biochim. Biophys. Acta* **125**, 474-484
- Benacerraf, B., Bilbey, D., Biozzi, G., Halpern, B. N. & Stiffell, C. (1957) *J. Physiol. (London)* **136**, 287-293
- Biozzi, G., Benacerraf, B., Halpern, B. N., Stiffell, C. & Hillemand, B. (1958) *J. Lab. Clin. Med.* **51**, 230-239
- Branch, R. A., Shand, D. G., Wilkinson, G. R. & Nies, A. S. (1973) *J. Pharmacol. Exp. Ther.* **184**, 515-519
- Brauer, R. W. (1963) *Physiol. Rev.* **43**, 115-213
- Bray, G. A. (1960) *Anal. Biochem.* **1**, 279-285
- Butler, T. C. (1961) *J. Pharmacol. Exp. Ther.* **134**, 311-319
- Dobson, E. L. & Jones, H. B. (1952) *Acta Med. Scand.* **144**, Suppl. **273**, 1-71
- Felts, J. M. (1965) *Ann. N. Y. Acad. Sci.* **131**, 24-33
- Fine, M. B. & Williams, R. H. (1960) *Am. J. Physiol.* **199**, 403-408
- Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957) *J. Biol. Chem.* **226**, 497-509
- Glende, E. A., Hruszkewycz, A. & Recknagel, R. O. (1975) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **34**, 742
- Gordis, E. (1969) *J. Clin. Invest.* **48**, 203-209
- Greenway, C. V. & Stark, R. D. (1971) *Physiol. Rev.* **51**, 23-65
- Grim, E. (1963) in *Handbook of Physiology* (Hamilton, W. F., ed.), sect. 2, circ. 2, p. 1439, American Physiological Society, Washington, DC
- Hasamura, Y., Teschke, R. & Lieber, C. S. (1974) *Gastroenterology* **66**, 415-422
- Israel, Y., Videla, L. & Bernstein, J. (1975) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **34**, 2052-2059
- Lowenstein, L. M. (1971) *J. Biol. Chem.* **246**, 629-632
- McElroy, W. T., Siefert, W. L. & Spitzer, J. J. (1960) *Proc. Soc. Exp. Biol. Med.* **104**, 20-23
- Paul, B. B. & Rubinstein, D. (1963) *J. Pharmacol. Exp. Ther.* **141**, 141-148
- Rao, K. S. & Recknagel, R. O. (1968) *Exp. Mol. Pathol.* **10**, 219-228
- Razzak, M. A. & Wagner, A. N. (1961) *J. Appl. Physiol.* **16**, 1133-1138
- Recknagel, R. O. & Glende, E. A. (1973) *CRC Crit. Rev. Toxicol.* **2**, 263-297
- Recknagel, R. O. & Goshal, L. (1966) *Lab. Invest.* **15**, 132-146
- Reynolds, E. S. (1967) *J. Pharmacol. Exp. Ther.* **155**, 117-126
- Saba, T. M., Elkins, J. P. & DiLuzio, N. R. (1966) *J. Reticuloendothelial Soc.* **3**, 398-414
- Spector, A. A. (1968) *Ann. N. Y. Acad. Sci.* **149**, 768-783
- Traiger, G. J. & Plaa, G. L. (1972) *J. Pharmacol. Exp. Ther.* **183**, 481-488
- Ugazio, G., Koch, R. R. & Recknagel, R. O. (1973) *Exp. Mol. Pathol.* **18**, 281-289
- Wilkinson, G. R. (1975) *Annu. Rev. Pharmacol.* **15**, 11-27
- Witte, C. L. & Witte, M. H. (1974) in *Hepatic Circulation in the Liver* (Baker, F. F., ed.), part A, pp. 11-54, Marcel Dekker, New York
- Zilversmit, D. B., Salky, N. K., Trumbull, M. L. & McCandler, E. L. (1956) *J. Lab. Clin. Med.* **48**, 386-391