The Action of Adrenal Hormones on Hepatic Transport of Triglycerides and Fatty Acids[1]

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THE TRANSPORT and metabolism of various lipids by the liver must, of necessity, be regulated by hormonal and neurogenic means in order to maintain "lipid homeostasis" in the intact animal. For the past few years, we have attempted to examine the transport and metabolism of triglycerides (TG) and other lipids by the liver, and to learn how the nutritional state of the animal (1), hormones (2,3), drugs and toxic agents, such as CCl_4 (4,5) affect hepatic lipid transport and metabolism. Recently, we have been concerned with the effects of adrenal hormones, both cortical and medullary, on the transport of fatty acids and triglycerides by the liver. In order to evaluate these hormonal actions on the liver without the multiple variables inherent in experiments on intact animals, we studied the transport and metabolism of triglycerides and fatty acids in the isolated, perfused rat liver obtained from adrenalectomized and normal male animals. In recent years it has become apparent that the catecholamines, epinephrine (E) and norepinephrine (NE), stimulate release of nonesterified fatty acids from fat depots (6,7) and raise the levels of plasma nonesterified fatty acid (NEFA) (7–11). In addition, stimulation of the nerve supply to adipose tissue was observed to increase the release of NEFA (12), whereas denervation increased the lipid content of the depot (13). The neuroendocrine system was further implicated as a regulator of NEFA transport, since it was reported that certain physical and psychological stimuli increased plasma levels of NEFA in man (14,15). These effects of catecholamines on plasma NEFA levels have been related causally to stimulation of lipolysis of adipose tissue glycerides. In order to ascertain whether the transport and metabolism of lipids by the liver was affected by catecholamines, we investigated the action of E and NE on the transport of fatty acids and triglycerides by the isolated, perfused rat liver. It was also desirable that we evaluate the role of the adrenal cortex in hepatic lipid metabolism. We observed that NEFA uptake, TG release and TG uptake were inhibited by the addition of catecholamines to the medium perfusing livers from normal animals. Furthermore, TG release by livers from adrenalectomized rats was severely restricted, but was returned to essentially normal levels by pretreatment of the adrenalectomized rat with cortisone.

Experimental

Normal male rats (Holtzman Co., Madison, Wisc.), weighing 250–400 g, maintained on a balanced ration and water *ad libitum*, were used as liver donors. Livers also were removed seven days after operation from adrenalectomized rats that had been receiving 0.9% NaCl instead of tap water for drinking purposes. Adrenalectomized rats that were treated with cortisone were kept postoperatively for seven days before administration of the hormone; thereafter they received three injections of 5 mg cortisone acetate, i.m., and were used on the morning of the tenth day. The perfusion medium consisted of defibrinated rat blood obtained from normal, fed, male rats (1), and diluted to three times its original volume with Krebs-Henseleit bicarbonate buffer, pH 7.4 (16). Various additions of palmitate-serum complex, drugs, substrates and hormones were made as indicated. The perfusion apparatus is depicted in Figure 1. In the studies with catecholamines, the liver was removed from the donor animal, placed in the perfusion apparatus, and an equilibration period was allowed to permit attainment of maximum hepatic perfusate flow rate. A constant infusion of *l*-NE (or E) bitartrate in 0.9% NaCl, or saline alone, was started 40 min after insertion of the liver into the perfusion system. The catecholamines had marked vasoconstrictor effects on the liver, and flow of perfusate through the liver was sharply reduced. This vascular effect, however, was inhibited by microgram quantities of phenoxybenzamine (dibenzyline; DBZ) (3). Five hundred μg phenoxybenzamine HCl in 0.9% NaCl, injected directly into the portal venous cannula, were sufficient to maintain the normal maximum flow rates through the liver during the constant infusion of NE or E. The DBZ, moreover, did not affect the metabolic actions of the catecholamines that were investigated, excepting ketone body production. The DBZ was injected routinely within a few minutes after the start of the catecholamine infusion. Twenty mg palmitic acid, as the fatty acid-serum complex (4), were added to the perfusate 10 min after the DBZ addition. At this time, maximal flow rates had been reattained.

In the experiments concerned with the effects of adrenalectomy and adrenocortical hormones on hepatic TG release, the palmitate-serum complex was added to the medium 20 min after the liver was placed in the perfusion system. Three min after addition of the fatty acid, perfusate samples were taken for the zero time analytical measurements. Fatty acids were estimated by the Trout modification (17) of the Dole procedure (9), using Nile blue as an acid-base indicator. TG were determined by the method of Van Handel and Zilversmit (18) after adsorption of phospholipids on silicic acid. Glucose was measured according to the procedure of Nelson (19). Ketones were estimated by the method of Michaels et al. (20) after oxidation in the apparatus described by Greenberg and Lester (21), and urea as described by Friedman (22). Radioactivity of the TG containing fraction was measured in a Tracerlab liquid scintillation counter (1).

Results

The effects of E and NE on the uptake of nonesterified fatty acids by the liver are indicated in Figure 2. Both catecholamines reduced the rate of uptake of the palmitate from the medium. The concn of palmitate used in these experiments was removed almost completely from the perfusate by normal livers

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EXPLODED VIEW OF LIVER PERFUSION APPARATUS

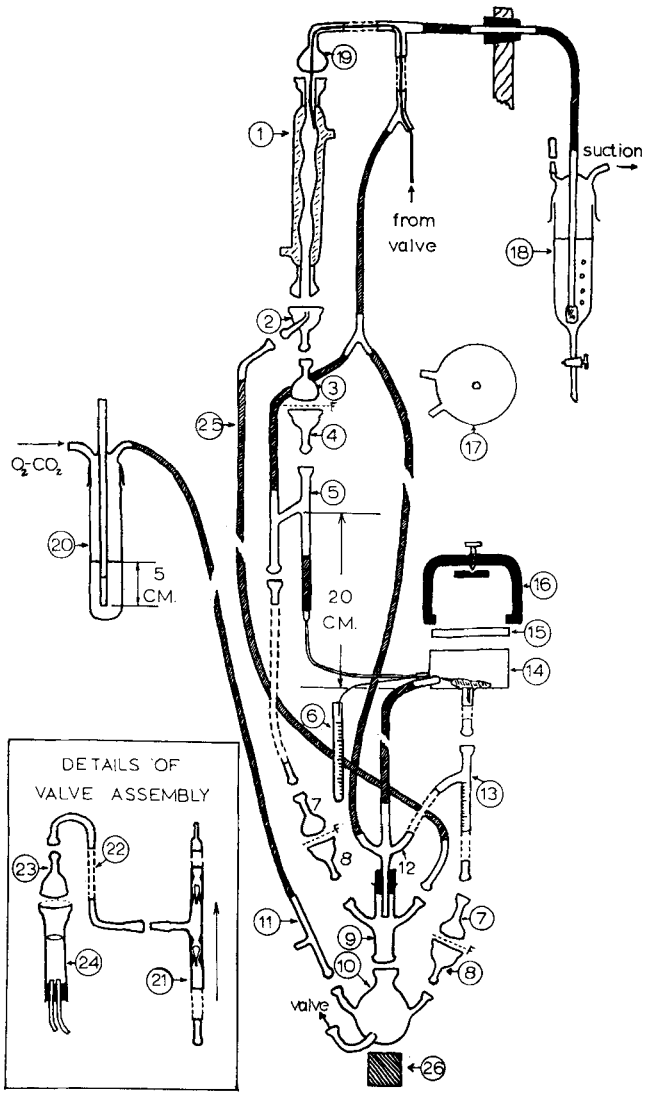

FIG. 1. *Liver Perfusion Apparatus.* All parts are glass except for liver chamber. J-S ball and socket joints, used throughout, allow easy assembly and cleaning. Parts are as follows:

(1) Condenser-aerator.
(2) Aerator connector.
(3) Filter half, top.
(4) Filter half, bottom.
(5) H-tube, for maintaining constant hydrostatic pressure.
(6) Bile collection tube, graduated.
(7) Filter half, top.
(8) Filter half, bottom.
(9) Chimney, to allow pressure equilization.
(10) Reservoir.
(11) Gas-flow connecting tube. Sidearm permits constant infusion of drugs, hormones, etc.
(12) Fork, connector for pressure equilization.
(13) Flow meter.
(14) Liver chamber, machined of lucite.
(15) Liver chamber top, O-ring seal.
(16) Liver chamber closing clamp, aluminum.
(17) Liver chamber, top view.
(18) CO_2 trap.
(19) Condenser connector.
(20) Manometer.
(21) Valve
(22) Valve-pump assembly connector.
(23) Pump assembly, head.
(24) Pump assembly, cylinder.
(25) Connecting tubing. Surgical grade rubber tubing is indicated by cross-hatching, tygon by dashed lines.
(26) Magnetic stirrer.

Entire apparatus is encolsed in a box maintained at 37C. Gas mixture from tank is passed through two gas washing bottles at 37C before entering manometer. Filter material F is 12 XX silk for screening, boiled and washed with distilled water before use. The entire system is closed. Suction is applied from water aspirator to pull gas phase through CO_2 trap. Negative pressure does not exceed −5 cm water. The reciprocal excursion of water in the pump assembly cylinder, by means of a Brewer automatic pipetting apparatus, is the driving force for lifting perfusate through the valve. In many recent experiments, the valve assembly was replaced by a peristaltic action pump (Model 500–1200, Harvard Apparatus Co.). Additional details may be obtained from the authors.

EFFECT OF EPINEPHRINE AND NOREPINEPHRINE ON NEFA UPTAKE BY THE LIVER

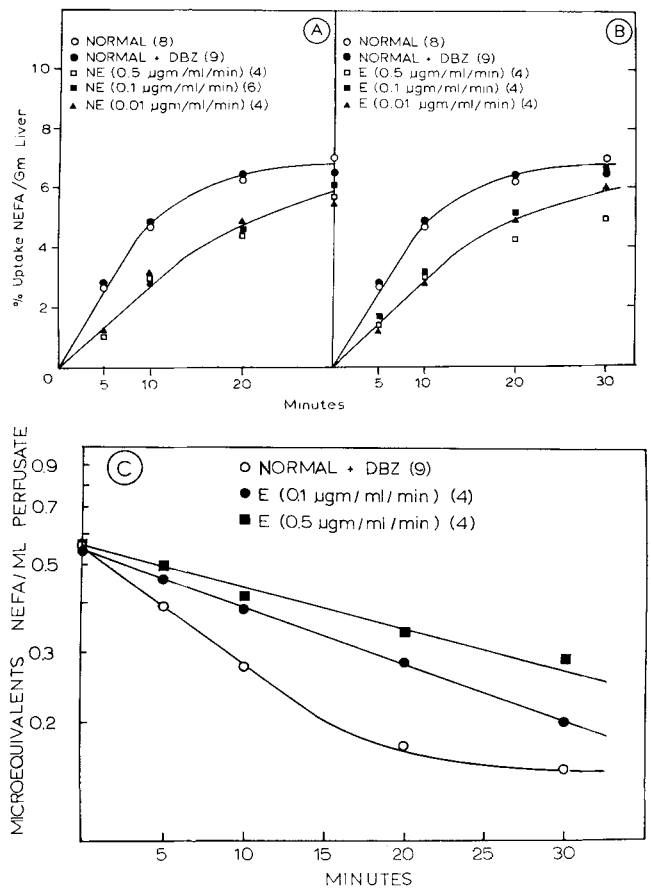

FIG. 2. *Effect of Norepinephrine (2A) and Epinephrine (2B) on NEFA Uptake by the Liver.* Infusion rates indicated for NE and E are calculated for free base. Numbers in parentheses in all figures and tables indicate number of experiments performed. The data as plotted in Figure 2C indicate that uptake of NEFA by the liver is a first order reaction. Means (% uptake NEFA/gm liver) ± standard deviation obtained 10 min following zero time sample:

1. Normal control	4.62 ± 1.57
2. Normal + DBZ	4.77 ± 1.02
3. E (0.5 μgm/ml/min) + DBZ	2.64 ± 0.99
4. E (0.1 μgm/ml/min) + DBZ	3.10 ± 0.15
5. E (0.01 μgm/ml/min) + DBZ	3.09 ± 1.03
6. NE (0.5 μgm/ml/min) + DBZ	3.00 ± 0.42
7. NE (0.1 μgm/ml/min) + DBZ	2.70 ± 0.71
8. NE (0.01 μgm/ml/min) + DBZ	2.94 ± 1.34

Statistical analysis: 1 vs. 2, NS, all other means differ from 2 with $p = 0.01$ or less.

within 20 min, and within 30–45 min by livers receiving the catecholamine infusion. It is of interest that the same degree of inhibition of NEFA uptake was observed with all three levels of administered catecholamines.

Effects of E on net TG release by the liver may be seen in Figure 3. Similar results were obtained with NE (3). E decreased the output of TG by the liver. It is apparent that DBZ had no effect on TG release or NEFA uptake, although it was very effective in blocking the catecholamine induced hepatic vasoconstriction.

NE, as well as E, appeared to increase the release of glucose by the liver (Table I). These observations confirm the glycogenolytic effects of these hormones on the isolated, perfused rat liver reported previously by Sokal et al. (23). Some of the incidental observations in these studies were an apparent stimulation of urea and ketone body production, and an inhibition of bile secretion induced by addition of the catecholamines to the medium (Table I).

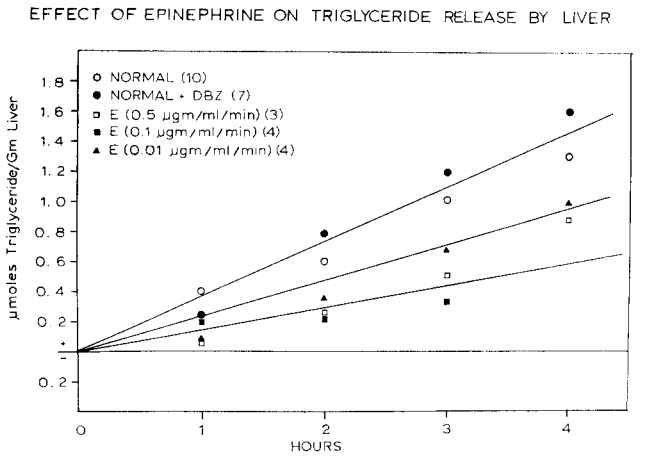

FIG. 3. *Effect of Epinephrine on Triglyceride Release by Liver.* Means ± standard deviation (3 hr data; μmoles TG/gm liver) are as follows:

1.	Normal	0.99 ± 0.54
2.	Normal + DBZ	1.21 ± 0.77
3.	E (0.5 μgm/ml/min)	0.52 ± 0.37
4.	E (0.1 μgm/ml/min)	0.33 ± 0.30
5.	E (0.01 μgm/ml/min)	0.68 ± 0.13

Statistical analysis:
1 vs.2, NS
2 vs. 4, $t_8 = 2.14$, $p = 0.036$
2 vs. 5, NS

E not only reduced TG release by the liver, but also inhibited the uptake by the liver of a neutral fat emulsion (NFE) labeled with tripalmitin-1-C^{14} (Fig. 4). E, infused at the rate of 0.1 μg free base/ml perfusate/min, reduced the rate of uptake of TG from the NFE. The disappearance of radioactivity from the medium is a more reliable index of uptake of TG from the NFE than is disappearance of chemically estimated glycerides, since the simultaneous release into the perfusate of nonradioactive, very low density lipoprotein TG by the liver adds lipid chemically indistinguishable by the Van Handel and Zilversmit procedure from the exogenous emulsion triglyceride. Thus, at lower TG concn, there actually seems to be a "reversal" of uptake of TG.

The change in relative specific activity of the perfusate TG with time is plotted in Figure 5. During the period that the perfusate emulsion TG is removed by the liver, it is diluted continuously by nonradioactive TG released by the liver, which results in a fall in the specific activity of the medium TG. E, perhaps by virtue of its inhibition of release of hepatic TG, retarded the drop in relative specific activity.

TABLE I

Effects of Catecholamines and Phenoxybenzamine on Production of Glucose, Urea and Ketones by Profused Liver

Analysis	Normal	Normal + DBZ	E + DBZ	NE + DBZ
	(A)	(B)	(C)	(D)
Glucose	16.82 ± 5.59 (5)	16.05 ± 5.49 (8)	41.62 ± 10.72 (4)	43.36 ± 6.31 (6)
Urea	0.82 ± 0.22 (8)	0.77 ± 0.14 (8)	1.03 ± 0.12 (4)	
Ketones	1.08 ± 0.58 (8)	1.66 ± 0.31 (8)	2.13 ± 0.42 (6)	
Bile flow	0.15 ± 0.03 (9)	0.15 ± 0.04 (7)	0.09 ± 0.03 (6)	0.06 ± 0.01 (6)

All figures are Means ± Standard deviation, and represent net changes in the perfusate. Values are expressed as mg glucose/gm liver, mg urea N/gm liver, mg acetone/gm liver, and ml bile/gm liver. All analyses, except glucose, were made 3 hr after addition of palmitate to the medium. Glucose values are net output from the time the liver was put into the perfusion system until 3 hr following palmitate addition (ca. 4 hr). Figures in parentheses indicate number of experiments. E or NE was infused at the rate of 0.1 μgm/ml/min.

Statistical Analysis:

Glucose	A vs. B, NS B vs. C, $t_{10} = 5.58$, $p = <0.001$ B vs. D, $t_{12} = 8.81$, $p = <0.001$
Urea	A vs. B, NS B vs. C, $t_{10} = 3.27$, $p = <0.01$
Ketones	A vs. B, $t_{14} = 2.49$, $p = 0.014$ B vs. C, $t_{12} = 2.41$, $p = 0.018$
Bile Flow	A vs. B, NS B vs. C, $t_{11} = 2.94$, $p = <0.01$ B vs. D, $t_{11} = 5.41$, $p = <0.001$

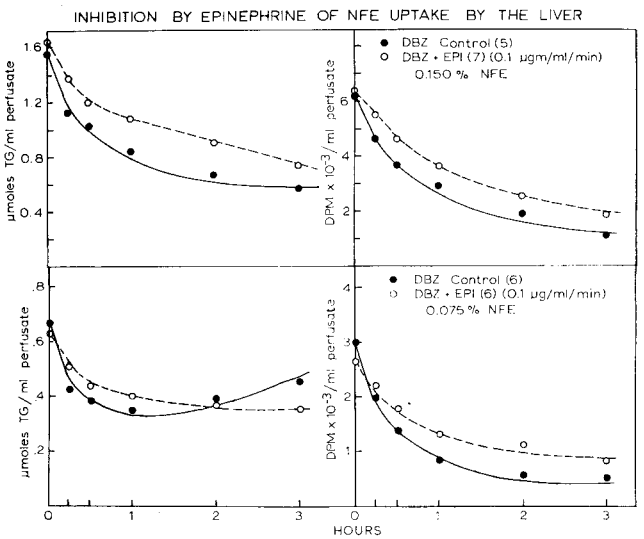

FIG. 4. *Inhibition by Epinephrine of Triglyceride Uptake by the Liver.* Percentage uptake DPM/gm liver is as follows:

Expt.	30′	60′
I. 0.075% NFE		
Control	5.68 ± 1.73	7.65 ± 1.88
E	3.29 ± 1.15	5.01 ± 0.91
	$t_{10} = 2.81$, $p = 0.01$	$t_{10} = 3.08$, $p = 0.01$
II. 0.150% NFE		
Control	4.62 ± 1.93	5.94 ± 2.39
E	2.79 ± 0.76	4.02 ± 0.66
	$t_8 = 2.14$, $p = 0.04$	$t_8 = 1.90$, $p = 0.04$

A neutral fat emulsion (Lipomul, labeled with tripalmitin-1-C^{14} was obtained from the Upjohn Co.) was added to the perfusate such that the initial concentration was 0.075% or 0.150% (w/v), respectively (See Ref. 1).

Additional TG uptake experiments were carried out in which twice the concn of NFE was present initially. The starting concn of NFE against rate of uptake by the liver is plotted in Figure 6, calculated over the first 30 min. During this time, the rate of uptake was relatively linear. It appears that uptake of TG was proportional to the concn, and that E inhibited the rate of uptake of the added TG (Fig. 6). We are currently examining the effects of E and NE on the uptake of chylomicron TG by the liver. In our past experience, however, this has not differed in any qualitative fashion from results obtained with the NFE.

Adrenocortical steroids appeared to have effects on net outward hepatic transport of TG in opposition to that of E and NE. TG output was depressed in livers from adrenalectomized rats (Fig. 7). In fact, a net decrease in perfusate TG concn was observed in experiments using livers from adrenalectomized animals. The net TG release by livers obtained from adrenalectomized, cortisone-treated rats approached that observed with livers from normal control animals. The addition of hydrocortisone to the medium *in vitro* did not increase the output of TG by the liver.

Discussion

In brief, the catecholamines E and NE appear to inhibit the uptake of fatty acids from a palmitate-serum complex and of TG from a NFE, and inhibit the release of TG from the liver. The inhibition of TG release in livers from adrenalectomized animals would thus appear to be primarily a result of cortical hor-

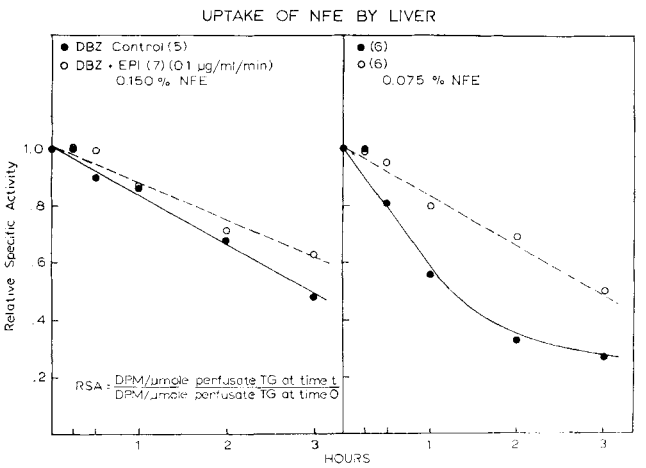

FIG. 5. *Uptake of Triglyceride by Liver.* DPM/µmole TG at time zero is set = 1. Composition of perfusate is as described for Figure 4.

mone deprivation, rather than loss of medullary hormones. It is of interest that whereas the catecholamines had a direct action on the liver, we have not as yet demonstrated any such action for the adrenal steroids.

The inhibition of TG release resulting from the addition of catecholamines to the medium cannot be the consequence of the decreased rate of uptake of palmitate from the medium, since in these experiments, the total fatty acid uptake was identical in the presence or absence of the hormones. The addition of palmitate to the medium stimulated the release of TG from the liver (4); it should be noted, however, that the uptake of NEFA appears to be a much more rapid process than is TG release. One of the conditions which may contribute to the E-induced decreased TG output is the increased ketone body production by the liver, a process which is indicative of increased fatty acid catabolism (24,25,26). E has been reported to inhibit the *de novo* hepatic synthesis of fatty acid (27), which then would, presumably, be less available for subsequent esterification and release as lipoprotein TG. It has been suggested that E increased the uptake of NEFA (portal-hepatic venous differences) by the liver of the intact animal (28,29). In the intact animal, however, we are dealing with a multiplicity of hormonal actions. The animal given E or NE responds with increases in plasma NEFA levels (7–11). This probably is due almost entirely to increased release of NEFA from adipose tissue depots, but may also be due, in part, to inhibition of uptake by liver. The increased levels of blood NEFA would make more fatty acid available to muscle and other tissues as an energy source (30). It is significant that the rate of oxidation of fatty acid by muscle was not stimulated by the addition of epinephrine *in vitro*, but rather appeared to be proportional to the fatty acid concn in the medium (30,31). If the uptake of NEFA by the perfused liver is proportional to the fatty acid concn (Fig. 2C) (32), then the observed increase of the portal-hepatic venous difference (28,29) may only reflect the elevated levels of plasma NEFA induced by E administration to the animal (33). This increased rate of NEFA uptake by the liver of the intact animal may be less than one might have expected with equivalent plasma NEFA levels but without the administration of E. It is pertinent that less stearic acid-1-C^{14} was recovered from the total liver fatty acids after the administration of E to rats than from untreated animals (34).

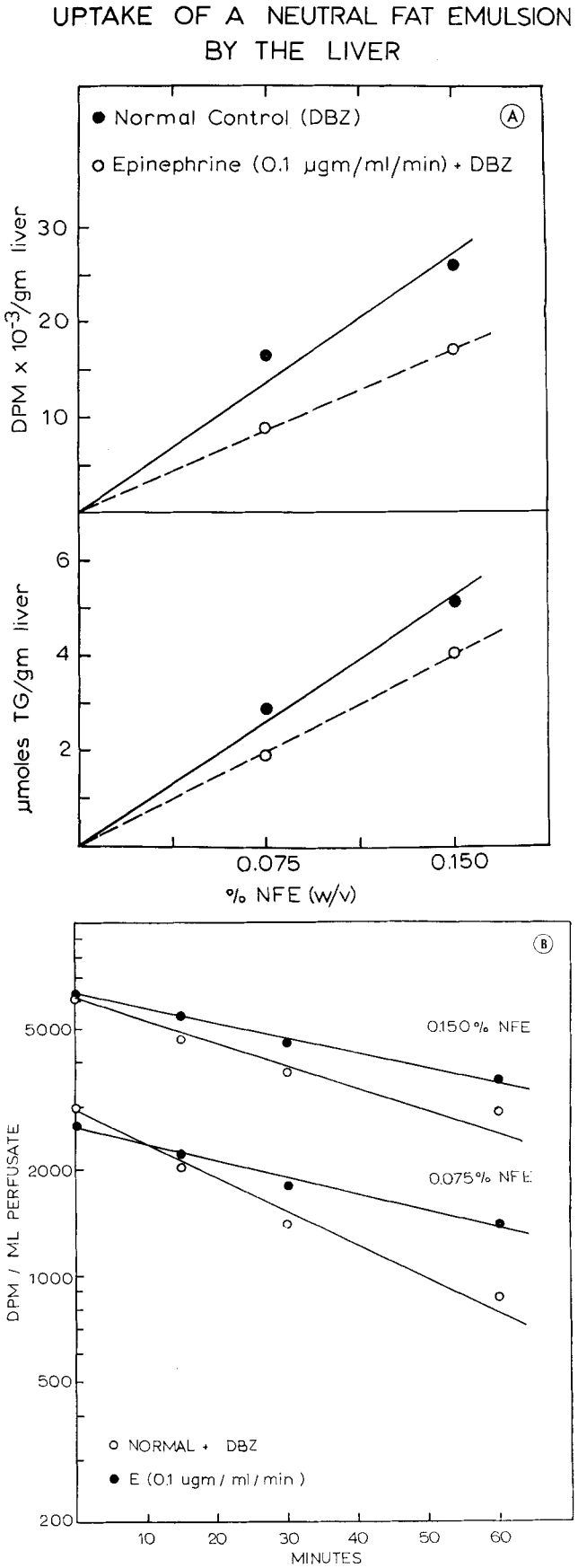

FIG. 6. *Uptake of Triglyceride by Liver.* Figure 6A indicates proportionality between rate of uptake of TG and concn of neutral fat emulsion in the medium. Data of Figure 6B (semilog plot of data from Fig. 4), and data from 6A, suggest that TG uptake by the liver is a first order reaction. Composition of perfusate is as described for Figure 4. Uptake of DPM/gm liver, ± SD, is as follows:

Expt.	30′
I. 0.075% NFE	
Control	16541 ± 4730
E	8754 ± 3720
	$t_{10} = 3.15$, $p = 0.005$
II. 0.150% NFE	
Control	26638 ± 10338
E	17481 ± 4520
	$t_8 = 1.97$, $p = 0.05$

Chronic administration of catecholamines to the animal has been reported to result in hyperlipemia and fatty liver (35). The increased NEFA in blood may be the source of the increased liver and plasma TG levels, in accordance with mass action effects. In addition, the inhibition of uptake of TG by the liver may contribute to an E-induced hyperlipemia.

The physiological effects of adrenocortical hormones on hepatic lipid transport and metabolism are very poorly understood. Much of the work in the past has concerned itself with the syndrome of fatty liver, which at best is a poor indicator of the intermediary metabolism of lipids, since it represents the summation of a number of concurrent processes. These processes include the uptake of NEFA and other lipids by the liver, the synthesis and oxidation of fatty acids, the esterification of NEFA to glycerides and phospholipids, and the outward hepatic transport of lipoprotein lipid. It has been known for some time that various stimuli which produce a fatty liver in normal animals will fail to do so in adrenalectomized animals (36). The lack of release of TG by the livers from adrenalectomized rats cannot be related to NEFA availability in any simple fashion since, in the experiments reported here, palmitate was added to the perfusion medium and appeared to be taken up by livers from both normal and adrenalectomized rats at essentially identical rates. Adrenal corticosteroids, added *in vitro,* stimulated the release of NEFA from adipose tissue isolated from normal and adrenalectomized rats (37). Mobilization of NEFA from adipose tissue of adrenalectomized rats has been variably reported as unchanged (38) or decreased (39,40). It does not seem likely that hepatic oxidation of fatty acid is increased following adrenalectomy. The production of $C^{14}O_2$ and acetoacetate from octonoate-1-C^{14} did not appear to differ in livers from normal and adrenalectomized rats (41). Ketosis is not readily produced in adrenalectomized animals (36), and furthermore, ketosis is depressed in both normal and adrenalectomized animals (42,43) and in man (44) upon corticoid administration. The ketogenic effect of the adrenal cortical steroids in diabetic animals (45,46) may be related to stimulation of NEFA release from adipose tissue and an increased availability of NEFA to the liver under circumstances of more rapid fatty acid oxidation resulting from the underlying insulin deficiency (47). The *de novo* hepatic synthesis of fatty acids was reported to be depressed by cortisone (48) and variously stimulated (49), unchanged (50) or depressed (51–53) by adrenalectomy. If NEFA are taken up by the liver from adrenalectomized animals, are oxidized at normal rates, are synthesized at a rate equal to or less than normal, how may one account for the failure of such a liver to accumulate TG and to release TG into the blood (Fig. 7) (54)? One possible explanation may be that the esterification of fatty acid with α-glycerophosphate in the synthesis of TG (55) is depressed in livers obtained from adrenalectomized animals. It is most interesting in this regard that the concn of α-glycerophosphate is decreased and

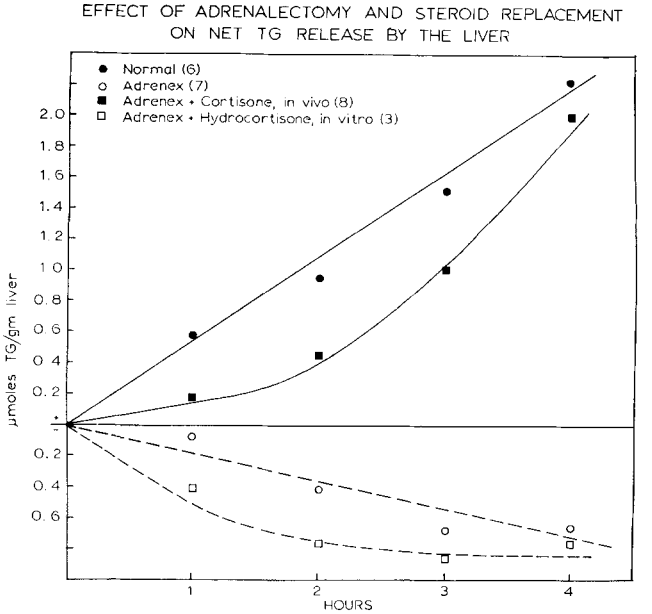

FIG. 7. *Effect of Adrenalectomy and Steroid Replacement on Net TG Release by the Liver.* Adrenalectomized animals were kept seven days postoperatively before use. Hydrocortisone monosodium succinate was added to the medium where indicated. A primer dose of 6.0 mg was added to the original perfusate (V = 130 ml, after NEFA addition); thereafter, the hydrocortisone was infused at a rate = 2.5 μgm/ml/min. Means ± standard deviation (2-hr data) are as follows:

1. Normal	1.00 ± 0.49
2. Adrenalectomized	−0.60 ± 0.24
3. Adrenex + cortisone (*in vivo*)	0.45 ± 0.29
4. Adrenex + hydrocortisone (*in vitro*)	−0.77 ± 0.30

Statistical analysis:
1 vs. 2, $t_{11} = 7.70$, $p < 0.0001$
1 vs. 3, $t_{12} = 2.65$, $p = 0.013$
2 vs. 3, $t_{13} = 7.60$, $p < 0.0001$
2 vs. 4, NS

that α-glycerophosphate dehydrogenase activity is increased in livers from adrenalectomized rats (56).

It is obvious that we are a long way from understanding the mechanisms by which these hormones affect lipid metabolism and transport, and so far we have raised more questions than obtained answers. The observation that adrenalcorticosteroids and adrenalmedullary catecholamines have opposite effects on net outward hepatic TG transport suggests as important homeostatic role of the adrenal gland in the regulation of the levels of plasma lipids and in the subsequent availability of lipids to extrahepatic tissues.

Summary

The cortical and medullary hormones of the adrenal gland have significant effects on the transport of TG and fatty acids by the isolated, perfused rat liver. E-inhibited TG release, as did prior adrenalectomy of the liver donor animal. Administration of cortisone to the adrenalectomized rat returned the TG release toward normal values. It would appear that adrenal cortical hormones stimulate TG release, whereas adrenal medullary hormones inhibit this process. Although the catecholamines inhibited TG output when added to the perfusion medium, administration of large amounts of hydrocortisone *in vitro* did not stimulate TG release by livers from adrenalectomized rats. One may surmise from these observations that the catecholamines act directly on the liver within a short time period; the adrenal cortical hormones, in contrast, either may require a longer period of exposure to the liver in order to exert a stimulatory action on

TG release, or may act indirectly in the intact animal via some mechanism, as stimulation of another endocrine organ. E and NE, in addition, inhibited the uptake of nonesterified fatty acid by the liver, and E also appeared to inhibit the uptake of TG from a neutral fat emulsion.

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• *Letters to the Editor*

Preparation of *a*-Monoglycerides

THE METHOD OF PREPARING *a*-monoglycerides based on the use of isopropylidene glycerol (1) has become a generally accepted procedure. The isopropylidene derivative is usually prepared by esterification of isopropylidene glycerol with fatty acid chlorides (2). Hartman (3) greatly reduced the time and work involved in the preparation of high purity monoglycerides by use of a method based on the esterification of isopropylidene glycerol with a free fatty acid.

This letter reports a further simplification of the technique for obtaining acyl isopropylidene glycerol utilizing standard laboratory apparatus. Instead of utilizing the aqueous phase collection apparatus suggested by Hartman (3), a medium sized Soxhlet extraction apparatus is used. The extraction thimble contains an appropriate amt of anhydrous magnesium sulfate, which is used to absorb the water formed during the esterification reaction (4). The reactants are placed in the boiling flask and refluxed for the required time. Isopropylidene glycerol can be obtained either by preparing it in the present apparatus by the condensation of acetone and glycerol catalyzed by *p*-toluene sulfonic acid by the method of Hartman (3), or by purchasing commercially available material (Aldrich Chemical Co.). Esterification is carried out by condensation of isopropylidene glycerol with the required free fatty acid using *p*-toluene sulfonic acid as a catalyst with alcohol-free chloroform or preferably with benzene as the carrier in order to remove the water formed during the reaction. For example, *a*-palmitoyl isopropylidene glycerol was prepared by refluxing a mixture of 0.14 mole palmitic acid, 0.44 mole isopropylidene glycerol and 0.02 mole *p*-toluene sulfonic acid dissolved in 300 ml benzene. The extraction thimble of the Soxhlet apparatus contained 0.3 mole of anhydrous $MgSO_4$. The reaction was virtually complete after four hr under reflux; its course was monitored with the aid of TLC on silica Gel G coated glass plates. The isopropylidene glycerol, free fatty acid, and isopropylidene ester were completely separated by a solvent system which was composed of

TABLE I

Fatty acid[a]	Time required for complete acylation of isopropylidene glycerol	Solvent	Yield of monoglyceride[b]	Purity of monoglyceride[c]
	(hr)		(%)	(%)
Lauric	3.5	benzene	98.5	98.9
Lauric	13	chloroform	63.5	97.4
Palmitic	4.5	benzene	87.0	98.0
Palmitic	16–21	chloroform	69.0	98.9
Stearic	3.5	benzene	80.0	98.9
Stearic	12	chloroform	76.0	97.4
Oleic	4.5	benzene	78.5	98.0
Oleic	7	chloroform	70.5	98.9
Linoleic	11	benzene	85.7	98.0
Linoleic	16	chloroform	78.0	98.0

[a] The purity of fatty acid employed here was 98% or greater.
[b] Yields presented are those obtained after recrystallization of the product. Yields prior to recrystallization were in the 90–98% range.
[c] Determined by the periodic acid method. Mehlenbacher, V. C., "The Analysis of Fats and Oils," Garrard Press, Champaign, Ill., 1960, p. 492.