Fatty Acid Synthesis in the Perfused Liver of Adrenalectomized Rats

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1. Fatty acid synthesis, measured in the perfused liver of fed adrenalectomized rats with $^3\text{H}_2\text{O}$ and ^{14}C -labelled precursors, was less than in control sham-operated rats. 2. This defect was more extensive for synthesis of fatty acids incorporated into triacylglycerols than into phospholipids. 3. There was impairment in desaturation and export of newly synthesized fatty acid. 4. Fatty acid synthesis and desaturation were restored to normal rates 5 h after treatment with cortisol *in vivo*. 5. Fatty acid synthesis was seasonally variable, being highest in the winter; the impairment after adrenalectomy was observed in all seasons. 6. In perfusions with oleate (0.7 mM), no further impairment in fatty acid synthesis was discerned in livers from adrenalectomized rats, in which the rate resembled that in control livers. 7. No defect in the incorporation of oleate into glycerides was discerned in livers from adrenalectomized rats. 8. Cortisol exerted no stimulatory effect on fatty acid synthesis when added to perfusion media. 9. The impairment in hepatic lipogenesis, demonstrable after adrenalectomy, shows that adrenal glucocorticoids promote hepatic capacity for fatty acid synthesis *de novo*, at least in intact non-diabetic rats. It is suggested that this effect is mediated by insulin, perhaps through direct action on the liver.

The role of adrenal corticosteroids in the regulation of the synthesis *de novo* of long-chain fatty acids in the liver is not clear. This matter is important, in view of the obesity which develops in people with Cushing's syndrome, and the changes in hepatic fatty acid synthesis which can occur in obesity (Hems *et al.*, 1975) and stress states (e.g. in a cold environment; Masoro, 1966). In previous attempts to shed light on this subject, by using adrenalectomized animals, fatty acid synthesis has been variously reported as diminished (Perry & Bowen, 1956), unchanged (Fain & Wilhelmi, 1962) or increased (Welt & Wilhelmi, 1950).

This problem is suitable for study with the perfused liver preparation. With the use of $^3\text{H}_2\text{O}$ to measure the total rate of conversion of acetyl units (from all sources) into fatty acid, conditions have been established, particularly with regard to circulating substrate requirements, which support optimal rates of fatty acid synthesis in the perfused liver (Salmon *et al.*, 1974). In the present study the role of corticosteroids in hepatic fatty acid synthesis has been investigated by using adrenalectomized rats. The present results suggest that adrenal glucocorticoids exert a positive influence on this process in the intact animal, probably through their action in promoting insulin secretion. A preliminary account of part of this work has already appeared (Kirk *et al.*, 1975).

Materials and Methods

Animals

Albino male Sprague–Dawley rats, weighing 180 g, were fed on a Thompson's (Heygate and Sons Ltd.,

Northampton, U.K.) supplemented cereal diet. Rats were bilaterally adrenalectomized 7–12 days before use. Diabetes was induced with streptozotocin (75 mg/kg; Whitton & Hems, 1975).

Chemicals

Chemicals were of the highest grade commercially available (for sources, see Elliott *et al.*, 1971; Salmon & Hems, 1973). Oleic acid and cortisol 21-succinate (sodium salt) were from Sigma (London) Chemical Co. (London S.W.6, U.K.). Digitonin was from BDH Chemicals Ltd. (Poole, Dorset, U.K.). Streptozotocin, from Upjohn Ltd. (Kalamazoo, MI, U.S.A.), was recrystallized from ethanol. Enzymes for enzymic assays were from C. F. Boehringer Corp. (London) Ltd. (London W.5, U.K.). Radioactive precursors were from The Radiochemical Centre (Amersham, Bucks., U.K.).

Perfusion of the liver

Livers were perfused with 50 ml of bicarbonate-buffered saline (Krebs & Henseleit, 1932) containing albumin (3.5%, w/v) and erythrocytes as described by Hems *et al.* (1972), except that anaesthesia was induced with diethyl ether. Being volatile, this anaesthetic would be removed from the liver in the initial phase of the perfusion. Glucose and lactate were added to the perfusion medium at initial concentrations of 15 and 10 mM respectively (Salmon *et al.*, 1974); their concentrations became approximately constant at 15 and 5 mM respectively (Glinsmann *et al.*, 1969). These concentrations are of the order found in the hepatic portal vein after oral glucose

administration, as used in the present experiments with intact rats (C. J. Kirk & T. R. Verrinder, unpublished work). The total rate of synthesis of fatty acids *de novo* was followed by measuring the incorporation into lipids (between 40 and 100 min of perfusion) of ^3H from $^3\text{H}_2\text{O}$ (2–4 mCi in each perfusion; Windmueller & Spaeth, 1966, 1967; Jungas, 1968; Brunengraber *et al.*, 1972, 1973; Salmon *et al.*, 1974). The rates of synthesis are expressed as C_2 units (acetyl residues) converted into fatty acids; the validity of this procedure has been discussed [see the references cited in the previous sentence; see also Ma & Hems (1975)]. The incorporation of ^{14}C from $[\text{U-}^{14}\text{C}]\text{-glucose}$ and $[\text{U-}^{14}\text{C}]\text{-lactate}$ provided a measure of lipid synthesis from these two precursors. In experiments involving the addition of $[\text{I-}^{14}\text{C}]\text{-oleate}$ to the perfusion medium, the fatty acid was bound to defatted bovine serum albumin (Chen, 1967; Krebs *et al.*, 1969); an initial dose of $[\text{I-}^{14}\text{C}]\text{-oleate}$ was added after 40 min perfusion, and more oleate was infused (12 ml/h) to maintain constant concentration (about 0.7 mM) and specific radioactivity (Topping & Mayes, 1972). Liver samples (median lobe), taken 1 h after the addition of labelled precursors, were frozen in liquid N_2 for extraction as described below. Perfusion medium was centrifuged briefly to obtain cell-free samples for lipid extraction, or extracted with HClO_4 for measurement of glucose and lactate (Hems *et al.*, 1972).

Measurement of fatty acid synthesis in intact rats

Total fatty acid synthesis *de novo* in intact rats (between 11:00 and 14:00 h) was measured by analysis of samples of liver and epididymal adipose tissue taken under diethyl ether anaesthesia and immediately frozen in liquid N_2 , 1 h after the injection of $^3\text{H}_2\text{O}$ and a trace dose of $[\text{U-}^{14}\text{C}]\text{-glucose}$ (Hems *et al.*, 1975). A sample of blood was collected at the time of liver sampling to measure the specific radioactivity of the plasma $^3\text{H}_2\text{O}$. In some experiments, animals were pretreated with 2 ml of 2 M-glucose intragastrically under light diethyl ether anaesthesia, 30 min before the injection of radioactive isotope; the aim of this procedure was to stimulate fatty acid synthesis, and perhaps reveal differences between groups.

Analytical methods

Samples of liver, adipose tissue or perfusate were either saponified directly in 20 vol. of 40% (w/v) KOH/ethanol (1:1, v/v) (Shigeta & Shreeve, 1964) or extracted with 20 vol. of chloroform/methanol (2:1, v/v) and washed by the method of Folch *et al.* (1957) before the separation of lipid classes by t.l.c. (Freeman & West, 1966). Phospholipids were eluted from the origin with chloroform/methanol/20 M- NH_3 (200:100:3, by vol.) and all other lipid classes were eluted with light petroleum (b.p. 40–60°C)/

diethyl ether/formic acid (50:50:1, by vol.). After elution from the various bands, the lipid classes obtained were saponified in 5 ml of 40% (w/v) KOH/ethanol (1:1, v/v) at 90°C for 3 h. Recovery of applied radioactivity from the t.l.c. plate was at least 90%. Free fatty acids were extracted from the saponified samples, as described by Brunengraber *et al.* (1973). In experiments where fatty acids were separated according to their degree of saturation, the fatty acids extracted from directly saponified livers were methylated in 14% (w/v) BF_3 in methanol for 5 min at 100°C (Metcalf & Schmitz, 1961), and extracted with light petroleum (b.p. 40–60°C). The fatty acyl methyl esters were then separated by silver-ion t.l.c. (Dunn & Robson, 1965), and eluted from the plate with diethyl ether.

The ^3H and ^{14}C radioactivities in the various samples were measured simultaneously by liquid-scintillation spectrometry (Salmon *et al.*, 1974) with the aid of a quench-correction technique (Hendler, 1964), which was adapted for computerized use. The specific radioactivity of the perfusate $^3\text{H}_2\text{O}$ was calculated (as d.p.m./g-atom of H in total H_2O) after measuring ^3H in cell-free perfusion medium and by assuming the perfusate water concentration to be 53 M. The initial specific radioactivity of the $[\text{I-}^{14}\text{C}]\text{-glucose}$ and $[\text{I-}^{14}\text{C}]\text{-lactate}$ were determined within 5 min of their addition to the perfusion medium. Lactate, glucose and glycogen were measured as previously described (Hems *et al.*, 1972).

Calculation of results

The total rates of fatty acid synthesis were calculated from the quotient (^3H as d.p.m. in lipid/g of liver)/(sp. radioactivity of $^3\text{H}_2\text{O}$). The isotope-discrimination effect for ^3H relative to ^1H was assumed to be 2.38 (Windmueller & Spaeth, 1966); since there are four H atoms per C_2 unit, the division of the above quotient by 1.7 yielded the total fatty acid synthesis, expressed as acetyl units. To calculate cholesterol synthesis, a factor of 1.4 was used (Windmueller & Spaeth, 1966, 1967). The incorporation of carbon from glucose and lactate into liver lipids was also calculated as acetyl units.

Results

The rates of fatty acid synthesis in the perfused liver of adrenalectomized rats were diminished compared with the rates in sham-operated controls or untreated animals (Table 1). Rates varied from 60 to 80% of those in sham-operated rats, depending on the season, there being a relative increase in all rates during the winter. There was an associated impairment in the appearance of newly synthesized fatty acid in perfusate, which amounted to 30–50% of that in controls, again depending on season (Table 1).

Table 1. *Synthesis of fatty acids in the perfused liver*

Livers of fed rats were perfused with bicarbonate-buffered saline containing albumin, erythrocytes, glucose (15 mM) and lactate (5 mM), as described in the text. $^3\text{H}_2\text{O}$ and ^{14}C -labelled lactate or glucose were added after 30 min, and the radioactivity in fatty acids was determined after a further 60 min. Total fatty acid synthesis was calculated from ^3H incorporation. Glycogen was determined in the right lobe, removed after about 10 min perfusion. For other details, see the text. Results are means $\pm$ s.e.m. for the number of observations indicated. In each group, total fatty acid synthesis in the livers or perfusates from adrenalectomized rats was significantly different from that in the sham-operated controls ($P < 0.01$, except in group 4, $P < 0.05$).

Experimental group	Type of rat	Season	No. of perfusions	¹⁴ C-labelled precursor	Total fatty acid synthesized (μmol of C ₂ units/h per g of liver)		Amount from ¹⁴ C-labelled precursor (%)		Initial glycogen (μmol of glucose/g fresh wt. of liver)
					Liver	Perfusate	Liver	Perfusate	
1	Adrenalectomized	Nov-Jan	9	Glucose	20.5 ± 0.9	2.1 ± 0.2	18 ± 1	18 ± 1	—
	Sham-operated	Nov-Jan	8	Glucose	28.7 ± 2.2	5.3 ± 0.9	19 ± 1	19 ± 1	—
	Adrenalectomized, treated with cortisol*	Nov-Jan	6	Glucose	11.0 ± 1.9	2.1 ± 0.6	10 ± 2	10 ± 2	—
2	Adrenalectomized	April	4	Glucose	19.2 ± 3.1	1.6 ± 0.1	13 ± 1	14 ± 1	—
	Sham-operated	April	6	Glucose	24.5 ± 1.0	3.3 ± 0.3	18 ± 2	16 ± 1	—
3	Adrenalectomized	July	6	Glucose	11.3 ± 1.4	0.7 ± 0.3	14 ± 1	22 ± 2	281 ± 44
	Sham-operated	July	6	Glucose	18.8 ± 1.8	2.4 ± 0.2	17 ± 1	19 ± 2	339 ± 27
	Untreated	July	5	Glucose	22.3 ± 1.5	3.4 ± 1.1	20 ± 1	19 ± 2	352 ± 28
4	Adrenalectomized	Sept	3	Lactate	12.6 ± 2.7	1.4 ± 0.4	28 ± 4	42 ± 10	302 ± 16
	Sham-operated	Sept	4	Lactate	21.4 ± 1.3	2.9 ± 0.4	29 ± 3	32 ± 8	332 ± 12

* Rats received cortisol (100 mg/kg) *in vivo* 2 h before perfusion.

Fig. 1. Restoration of fatty acid synthesis after treatment with cortisol

Adrenalectomized rats were treated with cortisol (10mg/kg, subcutaneously), and then fatty acid synthesis was measured in the perfused liver. The details of liver perfusions were as in Table 1 and the text. The period of ^3H incorporation was 40–100 min and the times shown for points on the Figure were calculated from the time of injection of cortisol (*in vivo*) to 70 min after the start of perfusion. The incorporation of C_2 units into fatty acid in liver (a) was measured as a total rate (●) and from glucose (■). Also, (b) export of newly synthesized fatty acid was measured by the ^3H in perfusate fatty acids at the end of perfusion (▲). In (b) is also shown the ratio of ^3H incorporated into fatty acids of triacylglycerols over that in phospholipids (▼). For each group, the value at zero time refers to untreated adrenalectomized rats, and the corresponding open symbol and broken line gives values for sham-operated animals. Results are means $\pm$ S.E.M. (vertical bars) from four separate perfusions for each point.

In livers perfused under the present conditions, fatty acids are synthesized mainly from glycogen, lactate, and to a lesser extent, glucose (Salmon *et al.*, 1974). An attempt was made to assess whether there was any specific impairment in lipogenesis from these precursors in the livers of adrenalectomized rats. The initial glycogen content of the perfused livers was similar in adrenalectomized, sham-operated and normal rats; thus there was no apparent shortage of glycogen in adrenalectomized group (Table 1). The rates of fatty acid synthesis from lactate and glucose were assessed with ^{14}C -labelled precursors. In the groups of perfusions shown in Table 1 (September), [^{14}C]lactate was present (in addition to $^3\text{H}_2\text{O}$). The lactate-carbon contribution to the total rate of fatty acid synthesis was 28% in the liver (Table 1), and about 37% for those in perfusate, in both sham-operated

and adrenalectomized rats. In the other groups of perfusions in Table 1, [^{14}C]glucose was present; the glucose-carbon contribution to total fatty acid synthesis was 10–22% (measured separately in both the liver and perfusate) in all groups. Thus in the perfused liver of the adrenalectomized rat, fatty acid synthesis from lactate or glucose was impaired to the same extent as was the total rate.

Two doses of cortisol were selected for testing with regard to the restoration of lipogenesis, from the reports of previous studies of similar type (e.g. Klausner & Heimberg, 1967). After pretreatment *in vivo* with cortisol (10mg/kg, subcutaneously) for 4–5 h, the capacity for fatty acid synthesis in the subsequently perfused liver was completely restored to normal, whereas complete restoration was not detected for the impaired export of newly synthesized fatty acid (Fig. 1). A larger dose of cortisol (100mg/kg) did not bring about restoration of fatty acid synthesis; rather, there was impairment of lipogenesis, from glucose in particular, after this treatment (Table 1).

The proportion of newly synthesized fatty acid that was present as monoenoic acids was decreased in the perfused livers of adrenalectomized rats, and restored to normal after pretreatment with cortisol *in vivo* (10mg/kg) for 5.2 h (Table 2).

Separation of lipid classes by t.l.c. (Table 3) indicated that whereas triacylglycerol fatty acid synthesis was consistently decreased in the perfused livers of adrenalectomized rats, an impairment in incorporation of ^3H into phospholipid fatty acid was not always observed (results from two seasonal groups in Table 3). The change in the relative rates of incorporation of newly synthesized fatty acids into the major lipid classes was not corrected by pretreatment (*in vivo*) with cortisol (Fig. 1). In these experiments, glucose contributed about 20% of carbon for fatty acid synthesis in both major lipid classes, and this contribution was not affected by adrenalectomy (results not shown). A preferential inhibition of triacylglycerol fatty acid synthesis was also detected in the perfused liver of streptozotocin-diabetic rats, in which fatty acid synthesis was markedly decreased (Table 3).

The glycerides in the perfusate were also separated by t.l.c.; 75–85% of the ^3H or ^{14}C in newly synthesized exported fatty acid was found in triacylglycerols, and no alteration in this proportion was observed in perfusate from livers of adrenalectomized rats (results not shown).

The above-described preferential inhibition of triacylglycerol synthesis suggested that the lesion in the liver of adrenalectomized rats could reside in processes whereby fatty acids are incorporated into triacylglycerols. This possibility was tested in perfusions including albumin-bound [^{14}C]oleate (Table 4). The incorporation of oleate carbon into lipids was not altered after adrenalectomy (Table 4). The total rate of synthesis *de novo* of fatty acid in livers from

Table 2. Distribution of ^3H and ^{14}C in saturated and monoenoic acids

Livers from fed rats were perfused (in April) with $^3\text{H}_2\text{O}$ and $[^{14}\text{C}]\text{glucose}$ as described in Table 1, or after treatment (*in vivo*) with cortisol (2mg) as in Fig. 1 and Table 3. Fatty acid methyl esters were separated according to saturation, by t.l.c. Data are expressed as a percentage of radioactivity recovered from the entire t.l.c. plate, which was at least 90% of that detected in total fatty acids (shown for ^3H , expressed as acetyl units converted). For other details, see the text. Results are means $\pm$ s.e.m. of three measurements in adrenalectomized rats and four in sham-operated rats. *P* values are given for each possible comparison (N.S., not significant).

Experimental group	Total fatty acid synthesis (μmol of C_2 units/h per g)	Radioactivity in fatty acid (%)					
		Saturated		Monoenoic		Di- and poly-enoic	
		^3H	^{14}C	^3H	^{14}C	^3H	^{14}C
1. Adrenalectomized	18.3 ± 3.6	90.3 ± 0.3	88.5 ± 0.1	4.1 ± 0.2	5.4 ± 0.2	5.6 ± 0.1	6.3 ± 0.1
2. Sham-operated	26.9 ± 1.3	85.2 ± 0.8	84.8 ± 0.3	10.8 ± 0.8	11.3 ± 0.7	4.1 ± 0.7	3.8 ± 0.6
3. Adrenalectomized, given cortisol 5.2h before perfusion	28.7 ± 2.6	81.9 ± 1.0	80.3 ± 1.3	9.9 ± 2.5	11.5 ± 2.4	8.2 ± 1.4	8.2 ± 1.3
<i>P</i> values							
1 versus 2	<0.02	<0.01	<0.001	<0.001	<0.001	N.S.	<0.02
2 versus 3	N.S.	N.S.	<0.01	N.S.	N.S.	<0.02	<0.02
1 versus 3	N.S.	<0.001	<0.01	N.S.	<0.05	N.S.	N.S.

sham-operated rats was less ($P < 0.001$) than in the absence of oleate (cf. Table 1), reflecting inhibition by oleate (Mayes & Topping, 1974). This rate resembled that in the liver of adrenalectomized rats perfused with oleate; thus the relative impairment in fatty acid synthesis in livers of adrenalectomized rats (compared with sham-operated animals) was not discernible in the perfusions with oleate (Table 4).

The possibility that glucocorticoids may exert a direct hepatic action on lipogenesis was tested in perfused livers. The results in Fig. 1 showed that long perfusions would be required to demonstrate any such effects. Therefore perfusions lasting 5h were carried out, in which cortisol was present throughout perfusion of one group of livers from adrenalectomized rats. The expected impairment in lipogenesis on adrenalectomy was observed (cf. Tables 5 and 1), but no stimulatory effect of cortisol on lipogenesis (during 5h) was detected (Table 5).

The impairment of hepatic fatty acid synthesis after adrenalectomy that was observed in the controlled conditions of liver perfusions was not apparent in the intact animal, whereas in adipose tissue (after a glucose load) fatty acid synthesis was decreased (Table 6). In these experiments *in vivo*, rats also received $[^{14}\text{C}]\text{glucose}$; the ratio $[^{14}\text{C}]/[^3\text{H}]$ (d.p.m.) in adipose tissue/ $[^{14}\text{C}]/[^3\text{H}]$ (d.p.m.) in liver then provides a measure of the relative significance of glucose in hepatic lipogenesis (Hems *et al.*, 1975). The ratio was 3–5, confirming the relatively minor role of glucose in hepatic fatty acid synthesis; no difference between adrenalectomized and control rats was detected.

In selected perfusions, cholesterol synthesis was

also measured by ^3H incorporation from $^3\text{H}_2\text{O}$. After adrenalectomy, there was no significant and consistently discernible alteration in cholesterol synthesis in livers perfused at two different seasons under the conditions of these experiments (Table 7); this confirms the general conclusion of Nervi & Dietschy (1974) obtained with liver-slice experiments. There was also no significant change in cholesterol synthesis in diabetic rats (Table 7); the decrease in glucose contribution presumably reflected the impaired hepatic glucose uptake, characteristic of diabetes.

Discussion

Hepatic fatty acid synthesis in adrenalectomized rats

These results, obtained in conditions which are optimal for hepatic fatty acid synthesis (Salmon *et al.*, 1974), demonstrate that there is a decreased hepatic capacity for fatty acid synthesis after adrenalectomy. The associated decline in the synthesis *de novo* of monoenoic acids after adrenalectomy provides another example of parallelism between lipogenesis and monoenoate synthesis (Gellhorn & Benjamin, 1966). After adrenalectomy, the impairment in lipogenesis is associated with a more marked decrease in the rate of export of newly synthesized fatty acid (and of net fatty acid release; Klausner & Heimberg, 1967). This would be expected, in view of the findings that the loss of lipogenic capacity after adrenalectomy applied particularly to triacylglycerol fatty acid synthesis, and that the major part of exported newly synthesized glyceride fatty acid (at

Table 3. *Distribution of radioactivity in liver glycerides*

In the groups of livers described in Table 1 and Fig. 1 and in livers of diabetic rats which received $^3\text{H}_2\text{O}$, triacylglycerol and phospholipid were separated by t.l.c., saponified, and ^3H and ^{14}C (from [^{14}C]glucose) determined in fatty acids. During this procedure, about 9% of radioactivity in fatty acid was lost during washing (Folch *et al.*, 1957) and another 14% during t.l.c., elution, saponification, and fatty acid extraction. Data are not corrected for these losses. For other details, see the text. Results are means $\pm$ s.e.m. Within groups 1 and 3, all differences between experimental and control rats are highly significant ($P < 0.01$).

Experimental group	Type of rat	Season	Cortisol (10mg/kg before perfusion)	No. of measurements	Total fatty acid synthesis (μmol of C_2 units/h per g)		Ratio		
					All classes (no Folch extraction)	Triacylglycerol fatty acid	d.p.m. in triacylglycerol fatty acid		^{14}C
							^3H	d.p.m. in phospholipid fatty acid	
1	Adrenalectomized	Nov-Dec	—	7	19.7 $\pm$ 0.9	4.8 $\pm$ 0.7	9.2 $\pm$ 0.8	0.52 $\pm$ 0.04	0.57 $\pm$ 0.05
	Sham-operated	Nov-Dec	—	4	32.0 $\pm$ 2.4	13.3 $\pm$ 1.2	13.1 $\pm$ 0.4	1.09 $\pm$ 0.11	1.19 $\pm$ 0.12
2	Adrenalectomized	Feb-Apr	—	3	19.2 $\pm$ 3.1	3.1 $\pm$ 0.6	8.7 $\pm$ 1.6	0.36 $\pm$ 0.04	0.40 $\pm$ 0.04
	Adrenalectomized	Feb-Apr	4.5	3	16.1 $\pm$ 2.1	3.1 $\pm$ 0.3	8.2 $\pm$ 0.7	0.38 $\pm$ 0.20	0.47 $\pm$ 0.02
3	Adrenalectomized	Feb-Apr	5.2	4	29.6 $\pm$ 3.1	4.8 $\pm$ 0.8	12.7 $\pm$ 2.9	0.39 $\pm$ 0.05	0.50 $\pm$ 0.05
	Sham-operated	Feb-Apr	—	6	24.6 $\pm$ 1.0	7.3 $\pm$ 1.0	8.7 $\pm$ 1.0	1.07 $\pm$ 0.36	1.33 $\pm$ 0.51
	Normal	July	—	4	29.8 $\pm$ 8.0	14.2 $\pm$ 1.6	12.5 $\pm$ 1.2	1.18 $\pm$ 0.17	1.34 $\pm$ 0.20
	Streptozotocin-diabetic	July	—	4	3.5 $\pm$ 0.6	0.8 $\pm$ 0.2	1.5 $\pm$ 0.3	0.50 $\pm$ 0.10	—

Table 4. *Lipogenesis in livers perfused with oleic acid*

Livers were perfused (during March and April) as described in Table 1, except that [^{14}C]oleic acid (0.7 mM in cell-free perfusate) was present, and that livers were analysed 30 min after radioactivity was added. Incorporation of C_2 units into fatty acid was measured with $^3\text{H}_2\text{O}$. For other details, see the text or Table 3. Results are means $\pm$ s.e.m. for four adrenalectomized and five sham-operated rats. With regard to the rates of synthesis *de novo* of fatty acid, differences between groups 1 and 2 are not significant, and those between group 3 and the other groups are all significant (as indicated).

Experimental group	Conversion of precursors into hepatic glyceride ($\mu\text{mol/h}$ per g of fresh liver)						Ratio		
	Oleate			C_2 units			d.p.m. in triacylglycerol fatty acid		^3H
	Total	Triacylglycerol fatty acid		Total	Triacylglycerol fatty acid		^{14}C		
		Phospholipid fatty acid			Phospholipid fatty acid				
1. Adrenalectomized	18.1 ± 2.9	7.6 ± 1.4	5.0 ± 0.9	16.4 ± 1.3	4.2 ± 0.6	5.6 ± 0.8	1.52 ± 0.08	0.75 ± 0.05	
2. Sham-operated	18.0 ± 1.9	7.2 ± 1.1	4.1 ± 0.6	15.9 ± 0.7	5.0 ± 0.7	4.6 ± 0.7	1.77 ± 0.18	1.07 ± 0.07	
3. Sham-operated*	—	—	—	24.6 ± 1.0	7.3 ± 1.0	8.7 ± 1.0	—	1.07 ± 0.36	
	<i>P</i> values								
1 versus 3	<0.01		<0.05		<0.02				
2 versus 3	<0.001		<0.01		<0.05				

* No oleate in perfusate; taken from Table 3.

Table 5. *Effect of cortisol on fatty acid synthesis in the perfused liver*

Livers from fed rats were perfused (in September) for 5 h with 100 ml of medium containing glucose (12 mM) and lactate (5 mM). In one group the perfusate also contained cortisol (10 μ g/ml initially, and then infused at 1.6 mg/h). After 4 h perfusion, $^3\text{H}_2\text{O}$ and [^{14}C]glucose were added. The radioactivity in total fatty acids was determined after a further 60 min. Other details were as described in Table 1. Results are means $\pm$ S.E.M. for the number of observations indicated. For all parameters, values in group 3 are not significantly different from those in group 1.

Experimental group	Number of perfusions	Fatty acid synthesized (μ mol of C_2 units/h per g)		Fatty acid synthesized from glucose (%)	
		Liver	Perfusate	Liver	Perfusate
1. Adrenalectomized	3	12.4 $\pm$ 2.6	2.4 $\pm$ 0.6	9.6 $\pm$ 1.5	15.5 $\pm$ 1.5
2. Sham-operated	3	19.4 $\pm$ 3.4	5.3 $\pm$ 1.0	11.8 $\pm$ 0.4	8.0 $\pm$ 2.1
3. Adrenalectomized, plus cortisol in perfusate	4	10.0 $\pm$ 2.0	1.7 $\pm$ 0.1	8.7 $\pm$ 1.6	13.1 $\pm$ 1.6

Table 6. *Synthesis of fatty acids in intact rats*

Male rats received $^3\text{H}_2\text{O}$ at about 11:00 h; ^3H in fatty acids was determined after 1 h. Some rats received glucose (2 ml of 2.0 M, intragastrically under light ether anaesthesia) 30 min before $^3\text{H}_2\text{O}$. For other details, see the text. Results are means $\pm$ S.E.M. for the numbers of observations in parentheses. Differences between adrenalectomized and sham-operated rats are not significant, unless indicated (* $P < 0.001$).

Experimental group	Season	Glucose treatment	Fatty acid synthesis (μ mol of C_2 units/g fresh wt. of tissue)	
			Liver	Epididymal adipose tissue
1a. Adrenalectomized	Jan-Mar	—	7.1 $\pm$ 1.7 (7)	3.5 $\pm$ 1.0 (7)
1b. Sham-operated	Jan-Mar	—	10.0 $\pm$ 1.6 (7)	5.3 $\pm$ 1.8 (6)
1c. Untreated	Jan-Mar	—	9.4 $\pm$ 1.0 (7)	10.5 $\pm$ 3.0 (6)
2a. Adrenalectomized	Sept-Oct	+	8.5 $\pm$ 2.2 (10)	4.3 $\pm$ 1.1 (10)*
2b. Sham-operated	Sept-Oct	+	10.5 $\pm$ 2.0 (12)	16.7 $\pm$ 3.8 (12)*

Table 7. *Cholesterol synthesis in the perfused liver*

In some of the perfusions described in Tables 1 and 3, cholesterol synthesis was followed by the incorporation of ^3H from $^3\text{H}_2\text{O}$ and ^{14}C from [^{14}C]glucose. For other details, see the text. Results are means $\pm$ S.E.M. for the numbers of observations indicated.

Experimental group	Season	No. of perfusions	Cholesterol synthesis (μ mol of C_2 units/h per g fresh wt. of liver)	
			Total	From glucose
1a. Adrenalectomized	Nov-Jan	8	4.1 $\pm$ 0.5†	0.82 $\pm$ 0.13†
1b. Sham-operated	Nov-Jan	10	2.5 $\pm$ 0.3†	0.43 $\pm$ 0.06†
1c. Adrenalectomized*	Nov-Jan	6	2.4 $\pm$ 0.6	0.19 $\pm$ 0.05
2a. Adrenalectomized	April	3	3.0 $\pm$ 0.5	0.36 $\pm$ 0.03
2b. Sham-operated	April	3	2.6 $\pm$ 0.3	0.32 $\pm$ 0.06
3a. Diabetic	July	4	2.3 $\pm$ 0.7	0.08 $\pm$ 0.04‡
3b. Normal	July	4	3.2 $\pm$ 0.6	0.35 $\pm$ 0.04‡

* Treated for 2 h *in vivo* with 100 mg of cortisol/kg.

† $P < 0.02$, group 1a versus 1b.

‡ $P < 0.001$.

least in these short-term experiments) was located in triacylglycerols.

The decreased capacity for fatty acid synthesis, demonstrable in perfused livers of adrenalectomized rats, could not be discerned *in vivo*. The present results, obtained by using $^3\text{H}_2\text{O}$ (the validity of which is discussed by Salmon *et al.*, 1974; Ma & Hems, 1975), therefore support a previous study in intact rats (Fain & Wilhelmi, 1962), and are in keeping with the failure of administered cortisol to influence hepatic lipogenesis *in vivo* (Volpe & Marasa, 1975). The inconsistency of results from such studies *in vivo* may be explained by the relatively small extent of the intrinsic change on adrenalectomy, shown here in the controlled conditions of liver perfusion, and by the fact that this impairment of capacity for fatty acid synthesis is seasonally variable. Another relevant factor is that use of ^{14}C -labelled precursors can lead to underestimates of total rates of hepatic fatty acid synthesis (Hems *et al.*, 1975). Nevertheless, a study with [^{14}C]acetate (Diamant & Shafir, 1975), in which cortisol was administered to rats, confirms our general view that glucocorticoids act to promote hepatic lipogenesis, at least in animals that are not diabetic or severely stressed. In parallel with this action, glucocorticoids stimulate the synthesis and export of triacylglycerol fatty acid (Klausner & Heimberg, 1967; Reaven *et al.*, 1974; the present work). This effect is presumably relevant to the enhanced synthesis and export of hepatic triacylglycerol in obesity, e.g. in genetically obese mice (Salmon & Hems, 1973; Hems *et al.*, 1975), in which there is hyperfunction of the adrenal cortex (Herberg & Kley, 1975). In Cushing's syndrome or stress states, these aspects of hepatic lipid metabolism have yet to be characterized.

The activities of fatty acid synthetase and acetyl-CoA carboxylase remain unchanged in livers of adrenalectomized rats, as reported by Volpe & Marasa (1975), who also found that the activities of these enzymes in adipose tissue were decreased after cortisol treatment (compared with control rats). This latter finding is at odds with our observation that fatty acid synthesis in the epididymal adipose tissue of adrenalectomized rats (pretreated orally with glucose) was impaired compared with that of sham-operated controls, but this result may merely reflect the impaired insulin response to glucose in adrenalectomized animals (Van Lan *et al.*, 1974). In contrast with the results of Volpe & Marasa (1975), administered cortisol has been reported to increase the hepatic activity of acetyl-CoA carboxylase (Diamant & Shafir, 1975), as has triamcinolone (Diamant *et al.*, 1975); these observations are compatible with the present results. A more puzzling finding is that adrenalectomy brings about an increase in the (low) rate of lipogenesis, or activity of acetyl-CoA carboxylase, in the liver of diabetic rats

(Linder & Migliorini, 1974; Volpe & Marasa, 1975); however, this could reflect a different (perhaps more complex) sequence of events during insulin deficiency (e.g. involving glucagon) rather than a direct hepatic response to lack of either insulin or glucocorticoids.

Although the hepatic capacity for fatty acid synthesis was restored 5 h after cortisol administration (*in vivo*), the relative rate of triacylglycerol (compared with phospholipid) fatty acid synthesis was not restored, and the capacity for export of newly synthesized fatty acid was not completely restored. Thus there may be additional lesions in hepatic lipid metabolism after adrenalectomy (e.g. in enzymes of 'esterification' pathways producing triacylglycerols, or processes of lipoprotein assembly). The appraisal of the role of glycogen, lactate and glucose in lipogenesis in adrenalectomized rats did not reveal any specific impairment in the routes for generation of pyruvate (for lipogenesis) from carbohydrate. The occurrence of normal glycogen contents in the livers of fed adrenalectomized rats contrasts with the major impairment in glycogen synthesis in starved adrenalectomized rats; this difference can be explained by changes in the concentration of plasma insulin, which appears to be implicated in the promotion by adrenal glucocorticoids of glycogen synthesis in the liver (Whitton & Hems, 1976).

The present results suggest then that the adrenal gland may have only minor significance in regulating the capacity for hepatic fatty acid synthesis, at least in unstressed animals such as those used in the present experiments. Nevertheless, the observed impairment in hepatic fatty acid synthesis after adrenalectomy demonstrates that adrenal corticosteroids can promote hepatic lipogenesis, at normal circulating concentrations. The functional significance of such an effect is not yet clear.

Possible role for insulin in the effect of glucocorticoids on hepatic lipogenesis

The restoration of the impaired fatty acid synthesis by cortisol (*in vivo*), if the dose is not excessive, establishes that glucocorticoids can promote fatty acid synthesis in the whole animal and also that the impairment on adrenalectomy could solely reflect glucocorticoid deficiency. It would not be surprising if corticosterone (the major circulating glucocorticoid hormone in rodents) can act like cortisol in this respect, as these two steroids closely resemble each other in structure and action. However, although cortisol treatment (*in vivo*) restored the total rate of hepatic fatty acid synthesis and desaturation in 5 h, there was no direct hepatic stimulatory action of cortisol (during perfusion over 5 h). The question therefore arises as to whether glucocorticoids (*in vivo*) act directly on the liver to influence fatty acid synthesis. Although Klausner & Heimberg (1967) found that high concentrations of cortisol could stimulate tri-

acylglycerol release from the perfused liver, our experiments (*in vivo*) showed that high doses of cortisol may not resemble normal concentrations in their action on fatty acid metabolism. Thus it remains possible that glucocorticoids do not normally influence fatty acid synthesis by direct hepatic action.

Rather, the inherent decline in the synthesis and export of fatty acids in the liver after adrenalectomy could be entirely due to insulin lack. This follows for two main reasons. (1) There is impaired insulin secretion, associated with lower plasma-insulin concentrations, in adrenalectomized rats (Van Lan *et al.*, 1974). Conversely, plasma-insulin concentration increases on glucocorticoid administration (Firth & Hales, 1968; Reaven *et al.*, 1974; Diamant & Shafir, 1975). (2) The characteristics of impaired fatty acid synthesis revealed in the present experiments resemble those which might be expected from mild insulin lack, e.g. with regard to the impaired export of triacylglycerol (Wilcox *et al.*, 1968; Basso & Havel, 1970) and production of monoenoic fatty acids (Mercuri *et al.*, 1974; Gellhorn & Benjamin, 1966). In this context it is relevant that insulin can promote hepatic lipogenesis and triacylglycerol export, partly through direct hepatic action (Topping & Mayes, 1972). Further, the impaired lipogenesis in both adrenalectomized and diabetic rats was particularly revealed by a decline in ^3H incorporation into triacylglycerols. The greatly decreased rates of fatty acid synthesis observed in streptozotocin-diabetic rats confirm many previous studies indicating that insulin lack results in a profound decline in hepatic fatty acid synthesis; thus it seems likely that mild insulin deficiency such as that found in adrenalectomized rats (Van Lan *et al.*, 1974) might cause impaired fatty acid synthesis of the degree observed. A similar conclusion, namely that glucocorticoids promote hepatic lipogenesis through hyperinsulinaemia, was reached by Diamant & Shafir (1975) from measurements of acetyl-CoA carboxylase. Also, promotion of hepatic glycogen synthesis *in vivo* by glucocorticoids may involve insulin action (Whitton & Hems, 1976).

It follows from the above considerations that other underlying characteristics of the impaired lipogenesis in adrenalectomized rats should resemble those in diabetic rats. For example, there have been many measurements of enzyme activities in diabetic or adrenalectomized rats. In some enzyme groups (e.g. those of gluconeogenesis) there are qualitative differences between these states. Changes reported in some hepatic enzymes of fatty acid synthesis after adrenalectomy (synthetase and acetyl-CoA carboxylase; Volpe & Marasa, 1975) are negligible. No reported data appear to be incompatible with the notion that the decline in hepatic lipogenesis, after ablation of the adrenal gland, reflects insulin lack. The same applies to the responses of enzymes of fatty acid synthesis to administered steroids (Diamant *et al.*,

1975; Volpe & Marasa, 1975), which could reflect insulin action (Diamant & Shafir, 1975).

The reported changes in enzyme activities in livers from adrenalectomized rats shed some light on the intrinsic impairment of hepatic lipogenic capacity which is present in this situation. For example, activities of citrate-cleavage enzyme (EC 4.1.3.8) (Lardy *et al.*, 1965) and enzymes of the hexose monophosphate shunt (Novello *et al.*, 1969) decline in the liver after adrenalectomy (as they do in diabetes; Lowenstein & Kornacker, 1964; Novello *et al.*, 1969). However, details of the enzymic response to adrenalectomy (which should qualitatively resemble that in diabetes, on the arguments advanced here) remain to be fully clarified. Such data might further explain the impairment in lipogenesis due to adrenalectomy.

In the perfusions with oleate, lipogenesis was similar in both adrenalectomized and sham-operated rats, i.e. oleate masked the impairment in fatty acid synthesis, observed in its absence. This suggests that the impairment in adrenalectomy could be partly due to an excess of hepatic free fatty acid (derived e.g. from excessive hepatic lipolysis), which is known to inhibit lipogenesis (Mayes & Topping, 1974). Such an explanation is in accord with the notion that insulin mediates the effect of glucocorticoids on lipogenesis *in vivo*, since insulin may specifically control lipolysis in the liver (Poledne & Mayes, 1970; Topping & Mayes, 1972).

Therefore, on the evidence so far, the moderate decrease in hepatic fatty acid synthesis, after adrenalectomy, could reflect a hepatic response to mild insulin lack. Similarly, restoration of hepatic lipogenesis and lipid export by cortisol could be mediated by insulin, perhaps through direct hepatic action (Topping & Mayes, 1972). More generally, these experiments show that glucocorticoids (at naturally occurring concentrations in plasma) and insulin act in the same sense, i.e. to stimulate hepatic fatty acid synthesis *de novo* (except perhaps in severely diabetic animals). Therefore it appears that in the (non-diabetic) whole animal, there can be no glucocorticoid-dependent 'insulin resistance' (i.e. antagonism of insulin effects) with respect to the process of hepatic fatty acid synthesis. Thus glucocorticoids could not be implicated in the decline in hepatic lipogenic capacity which ensues from insulin deficiency (Linder & Migliorini, 1974), whereas they are in the increase in gluconeogenesis (Exton *et al.*, 1973) and in the production of hyperketonaemia (Chernick *et al.*, 1972) during diabetes.

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