action of acid diphenylamine solution and phosphomonoesterase on deoxyribonucleic acid. Thirty-four components, consisting of oligonucleotides containing up to nine pyrimidine residues, have been separated and identified with respect to pyrimidine composition and chain length. Many of these, however, consist of mixtures of isomeric sequences in unknown proportions.

2. The amounts of these components in digests of calf-thymus deoxyribonucleic acid and herring-testis deoxyribonucleic acid have been measured. Many of the sequences were present in amounts significantly different from those expected on the basis of a random distribution of nucleotides in deoxyribonucleic acid, but, in accordance with earlier conclusions, no general trend common to both nucleic acids was seen.

3. Sequences of four consecutive deoxycytidylic acid residues were found in digests of deoxyribonucleic acid from calf thymus, herring testis. *Alcaligenes faecalis* and *Pseudomonas aeruginosa*. Sequences of five consecutive deoxycytidylic acid residues were not detected in any of these four preparations and therefore may be absent.

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Lipids of Human Adrenals

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Numerous studies have demonstrated that the adrenal gland is extremely rich in lipids, especially cholesterol, and that stress or administration of corticotrophin causes depletion of lipids, particularly cholesterol (Rogers & Williams, 1947; Adams & Baxter, 1949; Sayers, 1950). The range of conditions likely to cause 'stress' as used in this context is wide and includes severe infections, injuries, especially severe burns, exposure to cold and severe emotional upsets of the degree of severity associated with suicidal states.

Symington & Davidson (1956), Symington, Duguid & Davidson (1956) and Symington (1960), working with human glands obtained at autopsy

and from patients undergoing two-stage adrenalectomy for cancer of the breast, showed that stress or stimulation with corticotrophin caused disappearance of the sudanophil globules from the clear cells, which were replaced by compact cells.

There is now good evidence that cholesterol is utilized by the adrenal as a precursor of the steroid hormones (Hechter, 1958), but Grant (1960) has drawn attention to the fact that none of the schemes proposed envisages the use of cholesterol esters despite the evidence that these are involved.

It was clear that a detailed study of the lipids of the adrenal under varying conditions of stress or stimulation would be of value. In the present

study lipids were extracted from human adrenals obtained at autopsy and at two-stage surgical operations and were studied by chromatography on silicic acid. The sterol esters, triglycerides and phospholipids were hydrolysed and the fatty acids recovered were studied by gas chromatography.

An attempt has been made to correlate the results of these analyses with the different degrees of stimulation to which the glands had been subjected.

EXPERIMENTAL

Materials

Adrenal glands. Human adrenal glands were obtained at autopsy or at surgical operation. The post-mortem specimens were selected to include individuals who had been exposed to little or no stress before death and others who had been severely stressed.

The surgical specimens were obtained from three patients undergoing two-stage adrenalectomy for carcinoma of the breast. In each case the second operation was carried out 7 days after the first. Two of these patients (H and J) received 80 units of corticotrophin (Armour, Aotchar gel) intramuscularly 2 and 3 days respectively preceding the second operation and a further dose on the morning of the operation. In these three cases the right and left glands were examined separately, the former providing a relatively unstimulated control for the latter, which was exposed to the stress of the first operation and supplemented in the two cases mentioned by corticotrophin stimulation.

Solvents. All solvents were analytical grade except ethanol, which was technical-grade 'absolute alcohol'. This was rendered aldehyde-free by standing for several days over *m*-phenylenediamine with occasional shaking followed by distillation in an all-glass still.

Methods

All glands were treated in a similar fashion; the extracapsular fat was removed as far as possible, the gland was weighed and a small weighed block removed and fixed in neutral formalin for histological examination. The main portion was macerated in 50 ml. of water in a micro-macerator (Measuring and Scientific Equipment Ltd.) and the resulting emulsion poured into 250 ml. of methanol and allowed to stand overnight at room temperature. The liquor was filtered and the residue and filter paper were extracted again with 250 ml. of chloroform-methanol (1:1, v/v). This mixture was brought to the boil and filtered as before.

The residue and filter papers were finally refluxed for 3 hr. with 250 ml. of chloroform-methanol, filtered and the residue was washed with two 50 ml. portions of chloroform. The combined extracts and washings were evaporated to small volume at reduced pressure on the water bath at temperatures not exceeding 60°. The resulting aqueous emulsion was extracted three times with 100 ml. portions of light petroleum (b.p. 40–60°) and the combined light petroleum extracts were washed twice with water, dried over sodium sulphate, evaporated to dryness in a current of nitrogen on the water bath and stored at 0° under nitrogen until required for chromatography.

Silicic acid chromatography. Silicic acid (chromatographic grade, 200–300 mesh; L. Light and Co. Ltd.) was treated with concentrated hydrochloric acid to remove iron, washed with water, dried at 100°, cooled and washed twice with boiling acetone and twice with warm ether. Tubes of 18 mm. bore containing 26–50 g. of silica were used, giving columns 200–370 mm. long. Where smaller amounts of lipid were handled, columns of 10 mm. bore packed with 8.0 g. of silica to give a length of 160 mm. were used. The silica was transferred to the column as a slurry and finally packed by tapping the column with a metal rod whilst the solvent was allowed to flow. The most selective columns were obtained when the silica was made into a slurry and packed in ether and washed with 3 column volumes of light petroleum (b.p. 40–60°). All columns were operated at room temperature, usually about 20°.

The columns gave satisfactory separations with loadings of 50 mg. of lipid/g. of adsorbent, but in practice loadings did not usually exceed 10 mg./g. For a 26 g. column the crude lipid mixture, dissolved in 5 ml. of light petroleum, was applied to the top of the column and washed in with two further 10 ml. portions of light petroleum. Elution was commenced with 100 ml. of light petroleum, followed by 500 ml. of 1% (v/v) diethyl ether-light petroleum to elute the sterol esters. Triglycerides were eluted with 500 ml. of 6% (v/v) diethyl ether-light petroleum. Elution of free sterols was somewhat variable, possibly because of variations in different batches of silica; usually 15% (v/v) diethyl ether-light petroleum was effective but in some instances 25% of ether was required. Phospholipids were eluted by 250 ml. of chloroform-methanol (1:1, v/v) followed by 250 ml. of 100% methanol and finally 250 ml. of methanol to which had been added 2% (v/v) of water.

Fractions (20 ml.) were collected and portions (1 ml.) were removed and examined to follow the course of the chromatography. The Liebermann-Burchard reaction was used to follow the elution of the sterol esters and free sterols and the triglycerides were followed by the hydroxamic acid reaction (Hack, 1955). Phosphorus was determined by the method of Berenblum & Chain (1938); Martin & Doty's (1949) modification was useful for following the course of chromatography. Glycerol was determined by the method of Lambert & Neish (1950). Reversed-phase paper chromatography of sterols with phenyl-Cellosolve as the stationary phase was carried out by the method of Neher (Cook, 1958). The method of Baggett, Engel & Fielding (1955) was used for determination of sterol hydroxyl groups. Spot tests for phospholipids were used as described by Block, Durrum & Zweig (1955).

Hydrolysis. Sterol esters (approx. 100 mg.) were dissolved in 10 ml. of aldehyde-free ethanol, 5 ml. of 4*M*-sodium ethoxide and 2 ml. of water, and the mixture was refluxed for 3 hr. in an atmosphere of nitrogen. After cooling, water (10 ml.) was added and the sterols were extracted with three successive 10 ml. portions of light petroleum (b.p. 40–60°). The combined extracts were washed twice with water, the aqueous washings being added to the aqueous solution of soaps. The combined aqueous fractions were acidified with hydrochloric acid and the liberated fatty acids extracted with three successive portions of light petroleum. The combined light petroleum fractions were washed twice with water, dried over sodium sulphate and evaporated to dryness in a stream of nitrogen.

Triglycerides and phospholipids were hydrolysed in a similar fashion.

Methylation. Fatty acids were methylated by adding an excess of an ethereal solution of diazomethane. The ether and surplus diazomethane were evaporated at reduced pressure on the water bath.

Gas chromatography. The gas chromatogram was constructed in the laboratory. The carrier gas was argon and a ^{90}Sr β -ionization detector (Lovell, James & Piper, 1959) was used. Columns were of straight Pyrex tubing (3.5 mm. bore, 132 cm. long). The solid support was Celite 545 prepared as described by Orr & Callen (1959) and screened to 60–80 mesh. Stationary phases used were Apiezon L (Shell Chemical Co.) and polyethylene glycol adipate (James, 1959). The stationary phases formed 15% (w/w) of the total column packing. Apiezon columns were operated at 197°, polyethylene glycol adipate columns at 180°. Flow rates were of the order of 50 ml./min. Methyl esters were added to the top of the column with a micro-pipette: 80–100 μg . to Apiezon columns and twice that amount to columns of polyethylene glycol adipate. All mixtures of methyl esters were chromatographed on both types of column to determine fully the C_{18} acids.

Peaks were identified by comparison of retention times with published data (James, 1959; Ahrens *et al.* 1959; Stoffel, Insull & Ahrens, 1958; Hawke, Hansen & Shorland, 1959) and also by use of internal standards. Quantitative determinations were made by planimetry of peaks.

RESULTS

A pair of large adrenal glands (X, Table 2) was selected at autopsy for a preliminary survey of their lipid content. They weighed 11.59 g. and yielded 2.66 g. of an orange-coloured oil on extraction. A portion (766 mg.) of the crude extract was chromatographed on a column of silicic acid that had been packed in ether and washed with light petroleum. The elution pattern of the sterol esters is shown in Fig. 1. The material comprising the small peak 3 gave a fast Liebermann–Burchard reaction.

The three sterol ester fractions shown in Fig. 1 were examined separately. Hydrolysis was carried out as described by Riley & Nunn (1960) and the liberated fatty acids were titrated. The material comprising fraction 1 (49.5 g.) yielded approximately molar equivalence of fatty acids and cholesterol but fraction 2 (322 mg.) yielded 138 mg. of acid (by titration calculated as oleic acid) and only 173 mg. of unsaponifiable material (molar ratio fatty acid to sterol 1.09, calculated as cholesterol). Fraction 3 (42.5 mg.) yielded less than half the acid expected.

The unsaponifiable material from the three fractions dissolved in ethanol was examined by ultraviolet spectrophotometry. The material from fraction 2 showed only end absorption but fractions 1 and 3 absorbed strongly with maxima at 272 and 278 $\text{m}\mu$ respectively. The ultraviolet-absorbing material has not, however, been identified.

The possible presence of a sterol diol in fraction 2 was explored by reverse-phase paper chromatography of the unsaponifiable material. Two spots were obtained, one pink and possessing the same mobility as cholesterol and the other purple and slow-running. The latter features suggest a sterol diol but no diols were available at this time for comparison. Subsequently the unsaponifiable material obtained by the same technique from five other adrenal glands was examined. Three of four post-mortem glands and one gland obtained at operation gave similar slow-running purple spots.

A portion (87.5 mg.) of the unsaponifiable material from fraction 2 was acetylated by heating on the steam bath with acetic anhydride (1.0 ml.) and pyridine (0.1 ml.). The recovered acetates were chromatographed on a column of 10 g. of silicic acid and the elution pattern obtained is shown in Fig. 2. Fraction *E* proved to consist of cholesterol that had failed to become acetylated and fraction *C* was found to be cholesterol acetate. All the fractions were examined both by a quantitative Liebermann–Burchard reaction and by a quantitative hydroxamic acid reaction (Baggett *et al.* 1955), authentic cholesterol acetate being used as a standard. All fractions developed the expected amount of colour with the Liebermann–Burchard reaction but fraction *B* (11.7 mg.) gave twice as much colour by the ester determination as that given by a similar weight of standard (Table 1). Thus there was evidence to suggest the presence of significant amounts of a sterol diol in the adrenal gland.

The Rosenheim reaction, the selenium dioxide test (Fieser, 1951) and the Zimmermann reaction

Fig. 1. Separation of sterol esters from human adrenal glands on ether-treated silicic acid. (a) Light petroleum; (b)–(f) 0.25, 0.5, 1.0, 2.0 and 3.0% of ether in light petroleum. Fractions: 20 ml.

were carried out on the unsaponifiable material from fractions 1, 2 and 3 of the original crude lipid and were all negative. No precipitate was obtained with Girard T reagent but the material from

fraction 2 gave a slowly developing weakly positive Lifschutz reaction.

A portion (77 mg.) of the triglyceride fraction eluted with 6% (v/v) diethyl ether-light petroleum was hydrolysed, the liberated fatty acids were titrated and glycerol was determined on the hydrolysate. Glycerol (7.25 mg.) and 57.5 mg. of fatty acids were recovered, giving a ratio (with the acids calculated as oleic acid) 1:2.6.

Lipids from other glands were then fractionated on silicic acid columns. Table 2 shows total weights and total crude lipid content of all the glands examined together with details of their origin. Glands obtained at autopsy generally weighed more and contained more lipid than those removed at operation. This difference was not due solely to the disease from which the surgical patients were suffering since glands removed surgically for other conditions such as hypertension also are generally relatively small.

Table 3 shows the weights of the four main groups of lipids separated by silicic acid chromatography from seven pairs of adrenal glands obtained at autopsy. This series is arranged in order of increasing severity of stress, as determined by histological examination and also by consideration of the subject's immediate ante-mortem history. The last fractions eluted from the columns contained material that was apparently not phospholipid; the phospholipid was therefore estimated by determining the organically bound phosphorus in the combined fractions and multiplying by 25 (Borgström, 1951). It will be seen that cholesterol esters are least in the most highly stressed glands whereas free cholesterol is remarkably constant.

Table 4 shows the amounts of the four main lipid groups from the right and left adrenals of the three patients on whom two-stage adrenalectomy was performed, together with the comparable figures from a pair of glands (K) obtained at autopsy and treated in the same manner as the glands removed at operation.

Fig. 2. Separation of acetylated sterols from non-saponifiable material of fraction 2 (Fig. 1). (a) Light petroleum; (b) 0.5% of ether in light petroleum; (c) 10% of ether in light petroleum. Fractions: 7.5 ml.

Table 1. *Ester content of acetylated sterols after chromatographic separation*

Fractions B, C, E were obtained from peak 2 (Fig. 1) unsaponifiable material, separation of which is shown in Fig. 2. In column 3 the mol.wt. of the sterol is assumed to be 429.

Fraction	Wt. of fraction (as % of applied wt.)	Ester content (moles/mole of sterol)
B	11	2.02
C	49	1.09
E	13	0.25

Table 2. *Total weights and lipid contents of adrenal glands*

Source	Sex	Age (years)	Total wt. (g.)	Wt. of lipid (g.)	Diagnosis
X	M	60	11.59	2.659	Coronary insufficiency
A	F	80	11.25	2.41	Valvular disease of the heart
B	M	62	10.5	2.06	Coronary insufficiency
C	M	48	13.3	2.695	Coronary insufficiency
D*	M	69	5.85	0.602	Carcinoma of breast
E	F	53	10.4	1.895	Cerebral infarction
F	M	49	12.3	2.465	Coronary insufficiency
G*	F	41	6.58	1.370	Carcinoma of breast
H*	F	—	9.07	1.297	Carcinoma of breast
I	F	31	13.0	1.383	Cerebral haemorrhage during parturition
J*	F	69	7.02	0.983	Carcinoma of breast
K	F	32	9.64	1.273	Dissecting aneurysm of aorta
M	F	58	8.76	1.383	Haemorrhagic pneumonia

* Glands removed surgically.

These figures show a consistent pattern; in the left gland, removed at the second operation, the sterol esters are diminished whereas the phospholipids are increased. Triglycerides and free sterol are substantially unchanged. The changes in the left gland of case D, which was not stimulated with corticotrophin, are slight but are of a similar nature to those observed in the other two glands.

In two cases, portions of all fractions eluted after the sterol esters and up to the phospholipids

were titrated with 0.02N-tetramethylammonium hydroxide in aqueous 50% (v/v) ethanol, 0.1% bromothymol blue being used as indicator. No titratable acid was observed.

Table 5 shows the relative proportions determined by gas chromatography of the fatty acids in the sterol esters, triglycerides and phospholipids of the four pairs of adrenals obtained at autopsy. There is surprisingly little difference between lipids derived from different glands and such differences

Table 3. *Lipid contents and histological assessments of adrenal glands obtained at autopsy*

Histologica grading: severity of stress is indicated by +, ++, +++ (R), Reversion pattern (recovery from stress).

Source ...	Wt. of lipid (g.)						
	A	B	C	E	K	I	H
Fraction							
Sterol ester	0.950	0.750	1.225	0.608	0.631	0.487	0.207
Triglycerides	1.155	1.100	1.127	0.804	0.407	0.603	0.970
Free sterol	0.0789	0.076	0.0736	0.071	0.077	0.107	0.004
Phospholipids	0.046	0.082	0.078	0.118	0.017	0.219	0.060
Histological grading	(R)	+ to ++	++	(R)+	+ to ++	++ to +++	

Table 4. *Lipid contents and histological assessments of left and right adrenal glands*

For details of treatments of left (L) and right (R) glands removed at operation (D, H and J) and at autopsy (K) see text. Histological grading is described in Table 3.

Source ...	Wt. of lipid (g.)							
	D		H		J		K	
	R	L	R	L	R	L	R	L
Sterol esters	0.157	0.124	0.442	0.290	0.392	0.221	0.293	0.338
Triglycerides	0.085	0.080	0.128	0.147	0.084	0.103	0.131	0.276
Free sterol	0.013	0.011	0.017	0.017	0.017	0.013	0.035	0.042
Phospholipids	0.018	0.040	0.046	0.061	0.014	0.038	0.015	0.003
Histological grading	++	++	+	+++	+ to ++	++	+ to ++	+ to ++

Table 5. *Relative proportions of fatty acids from lipids of adrenal glands obtained at autopsy*

Source ...	Percentages of total wt.											
	Sterol esters				Triglycerides				Phospholipids			
	A	B	I	M	A	B	I	M	A	B	I	M
Acid												
Lauric	—	—	0.1	0.3	0.8	0.8	0.9	1.6	—	—	0.3	0.3
Myristic	2.5	3.4	4.2	2.8	9.1	5.3	5.0	7.2	1.7	3.8	2.9	1.6
Branched C ₁₅	0.5	0.6	0.9	0.5	0.6	0.5	1.2	0.9	0.1	1.5	0.4	—
Pentadecanoic	0.3	0.3	0.5	0.1	0.9	0.5	1.5	0.6	0.2	—	0.3	0.5
Palmitoleic	4.0	5.1	5.8	3.0	3.0	3.5	2.8	2.8	2.2	1.5	3.4	2.3
Palmitic	11.5	11.1	15.2	18.0	24.2	22.6	29.7	28.0	24.3	16.4	26.0	23.4
Branched C ₁₇	1.2	0.8	1.6	2.0	0.5	2.2	2.7	2.3	0.4	1.5	1.2	0.1
Heptadecanoic	0.3	1.7	0.3	0.2	0.8	1.1	—	—	0.3	1.1	1.1	0.3
Oleic	37.8	49.1	42.8	35.4	41.2	41.0	32.0	30.0	24.1	15.7	29.8	28.2
Linoleic	16.5	7.8	9.3	9.8	5.5	5.6	6.0	6.2	7.3	3.7	4.2	8.5
Stearic	9.5	7.6	9.6	10.5	12.5	10.0	6.0	12.0	25.7	27.6	17.0	25.4
Branched C ₁₉	—	—	2.4	7.7	—	3.0	1.3	3.4	—	1.9	—	—
Nonadecanoic	—	—	—	—	—	1.1	0.5	1.6	—	—	—	0.8
Arachidonic	2.5	2.0	0.9	1.9	0.9	0.8	1.8	1.3	7.5	13.0	10.1	5.2
C ₂₀ Trienoic	8.2	5.9	4.0	5.2	—	0.5	3.6	1.4	4.5	8.6	3.5	2.2
C ₂₀ Monoenoic	5.2	2.0	2.4	2.6	—	—	5.0	0.7	—	3.7	—	1.2
Arachidic	—	2.5	—	—	—	1.5	—	—	1.7	—	—	—

as do exist do not seem to be related to the stimulation of the glands. Oleic acid is the major component of the acids derived from the sterol esters, whereas linoleic acid is relatively small in amount and C_{20} triene is present in much greater quantities than arachidonic acid. More than 50 % of the acids of the phospholipids are saturated. Table 6 shows the relative proportions of fatty acids derived from the sterol esters, triglycerides and phospholipids recovered from the surgically removed glands. Differences between right and left adrenals are small and, for most acids, unrelated to the state of stimulation of the glands. In all three patients, however, the sterol ester linoleic acid was less and arachidonic acid greater in amount in the stimulated (left) than in the unstimulated (right) adrenal gland. In the triglycerides, linoleic acid was greater and stearic acid less in the stimulated than in the unstimulated glands, whereas the phospholipid stearic acid was greater in the stimulated glands.

DISCUSSION

The presence of a sterol diol in the adrenal gland is of some importance, but it could be an artifact. Bergström & Wintersteiner (1942) showed that oxidation of cholesterol in air commonly produces 7α - and 7β -hydroxycholesterol and 7-oxocholesterol, which were not found in the adrenal lipids, and furthermore when a synthetic mixture of lipids including cholesterol oleate and palmitate was subjected to the procedures used in this study no trace of a diol was found amongst the unsaponifiable material. It appears likely therefore that the diol reported here is a natural product.

The quantity of diol recovered, as determined by the hydroxamic acid reaction (Table 1), represented 11 % of the unsaponifiable material from fraction 2, whereas the quantity of acid recovered from hydrolysis indicated the presence of 9 % of doubly esterified diol. These findings agree in suggesting that about 8 % of the total sterol esters may consist of doubly esterified diol. The initial step in steroid biosynthesis requires the cleavage of the side chain of cholesterol to form isocaproic acid and the C_{21} steroid pregnenolone. Solomon, Levitan & Lieberman (1956) have produced evidence that 20-hydroxycholesterol is the intermediate. The finding of a sterol diol in the adrenal gland is not therefore unexpected but it is surprising to find that it is esterified.

Evidence that the sterol esters rather than the free sterol form the starting point for steroid biosynthesis is to be found in previous work (Popják, 1944; Adams & Baxter, 1949) and is supported by the findings in the comparative study described here.

In the post-mortem material the free sterol was remarkably constant in amount whatever the

Table 6. *Relative proportions of fatty acids from lipids of left and right adrenal glands*

The glands were obtained at surgical operation. L, Left; R, right.

Percentages of total wt.

Acid	Adrenals J											
	Adrenals D				Adrenals H				Adrenals J			
	Sterol esters		Triglycerides		Phospholipids		Phospholipids		Sterol esters		Triglycerides	
	R	L	R	L	R	L	R	L	R	L	R	L
Lauric	0.7	0.4	9.9	1.5	0.2	1.5	2.3	12.4	—	2.2	0.4	0.6
Myristic	4.5	3.2	3.7	6.6	2.9	5.4	7.4	5.6	1.6	2.0	2.6	2.5
Branched C_{15}	0.9	0.8	—	1.2	0.6	0.4	—	—	0.4	0.4	0.6	0.6
Pentadecanoic	1.0	0.9	—	0.9	1.8	1.9	—	—	0.3	0.2	0.3	0.5
Palmitoleic	5.6	4.1	6.2	3.7	1.8	2.7	3.5	3.6	4.6	4.3	4.6	5.8
Palmitic	13.2	8.8	30.2	24.6	21.8	29.0	23.4	19.4	10.6	12.7	22.0	23.6
Branched C_{17}	1.2	1.1	1.3	4.3	0.6	1.5	1.9	1.4	1.4	1.5	8.0	3.8
Heptadecanoic	0.6	0.4	0.5	0.9	0.4	0.8	1.4	0.6	0.2	0.6	0.7	1.0
Oleic	40.8	37.5	22.9	32.8	25.6	19.9	34.0	29.4	48.8	50.2	39.5	37.7
Linoleic	13.2	10.5	6.0	9.6	7.8	6.0	5.2	9.6	8.4	8.3	8.9	7.5
Stearic	7.4	7.1	15.1	8.4	24.2	24.4	12.2	9.8	4.8	5.6	8.9	6.5
Branched C_{19}	—	—	4.2	5.5	—	—	3.9	0.8	—	—	4.1	4.2
Nonadecanoic	—	—	—	—	—	—	0.9	0.6	—	—	0.5	1.3
Arachidonic	3.1	5.3	—	—	7.9	5.0	2.5	3.8	4.2	6.4	0.8	1.3
C_{20} Trienoic	4.1	10.5	—	—	3.0	1.5	1.4	3.0	8.0	5.7	0.4	1.5
C_{20} Monoenoic	3.7	9.4	—	—	—	—	6.7	2.9	6.8	2.0	0.6	1.0
Arachidic	—	—	—	—	1.4	—	—	—	—	—	0.6	0.6

amount of the sterol esters, whereas in the specimens removed surgically the sterol esters of the stimulated glands were depleted and the free sterol was unaffected. Hechter (1958) has calculated that the loss of cholesterol from stimulated rat adrenals is much greater than can be accounted for by the output of steroids. This contradicts the findings of Sayers (1950). In the present work losses of the order of 100 mg. of cholesterol occurred after 3 or 4 days' stimulation with corticotrophin. Since the daily output of steroids in the corticotrophin-stimulated human is considerably in excess of 25 mg. these losses are very modest and one must conclude that there is simultaneous replacement of sterol esters from the blood.

The increase in phospholipids that was noted in the stimulated glands has been noted previously during histochemical studies (T. Symington, personal communication). It is presumably a by-product of steroid biosynthesis but its significance is not understood.

The relatively small amount of linoleic acid in the adrenal sterol esters, especially in comparison with the blood sterol esters, in which cholesterol linoleate is a major component (Riley & Nunn, 1960), is the most notable feature revealed by gas chromatography. It has been shown that stimulation of the adrenal causes a loss of sterol esters from the gland (Popják, 1944), and since replenishment is most probably by the blood the small amount of linoleate suggests either that the adrenal accepts sterol esters selectively or, as seems more probable, utilizes them selectively. A little support is added to this statement by the fact that in the study of the surgically removed glands cholesterol linoleate was consistently slightly lower in the second-stage glands, but it must be emphasized that the differences between the two glands were slight and lay within the limits of error of the method used.

The presence of significant amounts of C_{20} triene is also of interest. The study of the glands removed surgically suggests that sterol ester arachidonic acid increases as the linoleate decreases when the glands are stimulated and Howton & Mead (1960) have shown that a C_{20} triene is an intermediate in the conversion of linoleate into arachidonic acid. The association in this case may be coincidence but it requires further investigation.

The triglyceride fatty acids show slight variation between individuals but are very similar to those found in human depot fat by Kingsbury, Paul, Crossley & Morgan (1961). It seems likely therefore that the adrenal triglycerides form part of the depot fat of the body. The significance of the increase of linoleic acid and decrease of stearic acid in the triglycerides is at present obscure.

The method of analysis used here involves

certain errors. First, the method of application of the sample to the column permits the escape of part or all of the volatile short-chain acids from C_{10} downwards, and it should be noted that no acid of chain length shorter than C_{12} has been observed in this study. Secondly, and more important, acids of chain length greater than C_{20} are strongly retained and only eluted after long periods. With overloading of the column and operating at 210° the sterol ester acids produced an additional peak, which was tentatively identified as a C_{22} polyene, but quantitative estimations were not possible. It is likely that acids of even longer chain length are present but undetected.

SUMMARY

1. The lipids from a series of human adrenal glands obtained at autopsies and surgical operations were fractionated by chromatography on silicic acid.
2. The lipid fractions from one pair of adrenals were examined in some detail. Evidence was found of the presence of a sterol diol.
3. Evidence is presented that when human adrenal glands are stimulated the sterol esters are depleted and the phospholipids increased. Free sterols and triglycerides are not appreciably changed.
4. The sterol ester acids differ appreciably from those found in blood, particularly in the small amount of linoleic acid.
5. It is suggested that the first stage of steroid biosynthesis takes place whilst cholesterol is still esterified.

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Phospholipid Interrelationships in Rat Epididymal Tissue and Spermatozoa

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Spermatozoa acquire their capacity to fertilize during their passage along the excurrent duct from the testis (see Hammond, 1941), and in some mammals at least they retain their fertilizing power for considerable periods of time while in this duct. The capacity of guinea-pig spermatozoa to cause fertilization is stated by Young (1931) to be substantially increased during their passage from the head to the tail region of the epididymis. In the fowl, on the other hand, Munro (1938) showed that spermatozoa do not acquire this capacity until they reach the level of the vas deferens. Munro pointed out that the rate of passage of spermatozoa through this duct is much greater in birds than it is in mammals and drew attention to the probability that the so-called 'ripening' of spermatozoa in the fowl is not acquired as part of an aging process as it could be in mammals. During this process of maturation it is difficult, however, to detect any change in the morphology or biochemistry of the spermatozoa recovered from different regions of the epididymis. One possible cytological change is the migration of the protoplasmic or kinoplasmic droplet from the neck of the mid-piece of the spermatozoa; it has been suggested that this

droplet (see Mann, 1954) is concerned with the nourishment of the spermatozoa. However, the region within the epididymis in which the migration of the droplet occurs varies in different species. Thus, whereas in the bull it occurs in the head of the epididymis, in the boar it occurs commonly in the body and even in the tail of the epididymis (Hancock, 1957). Other physical changes such as increase in specific gravity and in light-reflecting power of bull spermatozoa within the epididymis have also been observed (see Mann, 1954).

The finding that glycerylphosphorylcholine is copiously secreted in the epididymis (Dawson, Mann & White, 1957; Dawson & Rowlands, 1959; T. W. Scott, R. G. Wales, J. C. Wallace & I. G. White, unpublished work) suggests that it may be concerned in the maturation process (Bishop, 1961).

Since, in tissues such as the liver, glycerylphosphorylcholine is formed through the degradation of lecithin (Dawson, 1955), it was decided to examine the interrelationships of glycerylphosphorylcholine and lecithin in the rat epididymis. During these investigations it became apparent that there are differences between the concentrations of phospholipids in the head and tail regions of this organ.