to a second crop of crystals carrying more of the above. Vitamin A remains in the methanol-soluble fraction.

3. Chromatography on alumina allows the separation of ubiquinone from the cholesterol.

4. Acetylation of the sterol fraction followed by chromatography allows the separation of substance SC.

5. Ubiquinone has been found in normal liver of ten mammalian species in amounts from 10 to 40 $\mu\text{g./g.}$ Pullet liver was richer. Substance SC was not often detected and then only in very small quantities.

Part of the cost of the work was defrayed by a grant from the Nuffield Foundation and one of us participated as holder of an Agricultural Research Council Scholarship. We are indebted to Dr J. S. Lowe for valuable help.

A Study of 'Unsaponifiable' Extracts Obtained from Hydrolysates of Pancreas and Spleen

BY R. A. MORTON AND NAZEK I. FAHMY

Biochemistry Department, The University of Liverpool, Liverpool 3

(Received 27 August 1958)

The primary object of the present work was to extend to pancreas and spleen a survey of quantitatively minor constituents of tissue unsaponifiable fractions. In particular it was desired to ascertain whether ubiquinone (SA) (Festenstein, Heaton, Lowe & Morton, 1955; Heaton, Lowe & Morton, 1955, 1957; Morton, Wilson, Lowe & Leat, 1957) and α -tocopherol were present. In the course of the work a strongly adsorbed constituent was encountered and designated substance 'SF' until it was eventually identified as indole-3-aldehyde (3-formylindole) or a substance giving rise to 3-formylindole. The question arises whether this formylindole was an artifact derived from tissue proteins or some more specific precursor. Reference is made in the Discussion to the occurrence of 3-formylindole in products of biological interest.

The results of investigating pancreas and spleen from healthy animals made it desirable to study human tissue in a preliminary way. Ubiquinone and 3-formylindole have been obtained from human pancreas, and both substances, together with α -tocopherol, were found in human spleen. Unlike the cholesterol obtained from ox, sheep and pig spleen, which contained 7-dehydrocholesterol, the sterol from human spleen did not contain enough to be detected.

REFERENCES

- Cain, J. C. & Morton, R. A. (1955). *Biochem. J.* **60**, 274.
 Festenstein, G. N., Heaton, F. W., Lowe, J. S. & Morton, R. A. (1955). *Biochem. J.* **59**, 558.
 Heaton, F. W., Lowe, J. S. & Morton, R. A. (1955). *Biochem. J.* **60**, xviii.
 Heaton, F. W., Lowe, J. S. & Morton, R. A. (1957). *Biochem. J.* **67**, 208.
 Kantiengar, N. L., Lowe, J. S., Morton, R. A. & Pitt, G. A. J. (1955). *Biochem. J.* **60**, 34.
 Lowe, J. S., Morton, R. A. & Harrison, R. G. (1953). *Nature, Lond.*, **172**, 716.
 Morton, R. A., Wilson, G. M., Lowe, J. S. & Leat, W. M. F. (1957). *Chem. & Ind.* **133**, 275.
 Morton, R. A. & Fahmy, N. I. (1959). **72**, 99.
 Ward, R. J. & Moore, T. (1955). *Biochem. J.* **59**, xv.
 Williams, T. I. (1948). *An Introduction to Chromatography*, p. 13. London: Blackie and Son. Based on Brockmann, H. & Schodder, H. (1941). *Chem. Ber.* **74**, 73.

EXPERIMENTAL

Materials. Fresh pancreas and spleen from ox, sheep and pig were obtained from the Stanley Abattoir, Liverpool. The tissues were freed from adhering fat. In some experiments the lymph glands embedded in pancreas were excised and studied separately. Sheep pancreas was studied in some detail. The organ weighed from 36 to 53 g. (average of seven, 43 g.). Each contained oval pink glands, some small and some as big as a large bean. The glands varied in number from three to twelve (average eight) per pancreas. We have not found any references in the literature to these glands in sheep nor have our veterinary colleagues.

Human pancreas and spleen were obtained from the Department of Pathology, University of Liverpool, through the courtesy of Professor H. L. Sheehan. The tissue was obtained within about 12 hr. of death. It was at once cut into small pieces and saponified or left under ethanol at -20° overnight. Details of the three human spleens studied are: (i) Male, 41 years. Glioblastoma. Weight of spleen 99.9 g., 'unsaponifiable material' 0.506 g. (ii) Male, 55 years. Carcinoma of right suprarenal, metastases to liver, Cushing syndrome, severe hypokalaemia and hypocalcaemia. Weight of spleen, 121 g., 'unsaponifiable material' 0.395 g. (iii) Male, 74 years. Rheumatoid arthritis. Weight of spleen, 190 g., 'unsaponifiable material' 0.993 g.

Saponification. The following procedure (compare Tosio & Moore, 1945) tends to conserve tocopherols and ubiquinone. The tissue is cut into small pieces and weighed.

Pyrogallol (1 g./80 g. of tissue) is dissolved in ethanol (1 ml./2 g. of tissue) and added to the tissue. The mixture is quickly raised to the boiling point on a water bath. Potassium hydroxide solution (60%, 0.5 ml./g. of tissue) and more ethanol (1 ml./2 g. of tissue) are then added. Heating is continued for 20–45 min. After cooling, water (3 vol.) is added and the solution extracted with freshly redistilled ether, or occasionally with light petroleum. The bulked extracts are washed, dried and the solvent is removed. The unsaponifiable matter may be dissolved in hot methanol and cooled. The cholesterol which crystallizes is filtered off.

Chromatography. Alumina (grade O, P. Spence and Co., Widnes) was washed with acid, dried and partially deactivated to Brockman grade III (Williams, 1948).

Solvents. Light petroleum (b.p. 40–60°) was dried over sodium wire and distilled. Ether was usually dried similarly and distilled over reduced iron immediately before use.

Efficacy of pyrogallol in the saponification process. The effectiveness of pyrogallol as an antioxidant for known compounds such as vitamin K₁ and α -tocopherol was tested. It was reasonable to assume that experimental conditions allowing their recovery after refluxing with alkali would also favour conservation of ubiquinone and α -tocopherylquinone.

When menadione (2-methyl-1,4-naphthoquinone) was added to fatty material subjected to saponification it was not recovered; it had in fact formed a violet compound. The 2,3-disubstituted compound vitamin K₁ did not behave in this way. Provided that the time of saponification was not too long and that pyrogallol was added, both vitamin K₁ and α -tocopherol could be recovered to the extent of 85–90% after chromatography on acid-washed alumina.

Separation of ubiquinone and α -tocopherol. These two substances are eluted from acid-washed alumina together. If the fraction (freed from solvent) is treated with methanol at –15° or –25° the α -tocopherol is readily soluble. Under favourable conditions a very clean separation can be achieved.

RESULTS

Pancreas

Ox pancreas. Five batches of tissue (2.16 kg. in all) were used. In a typical experiment 343 g. of tissue gave 0.87 g. of 'unsaponifiable material' from which 0.35 g. of cholesterol was removed,

leaving 0.52 g. to be chromatographed. Light petroleum eluted a red oil containing a little carotene. Saturated hydrocarbons (50 mg.) and cholesterol (257 mg.) were the main products. Ubiquinone and α -tocopherol were eluted by 2–4% (v/v) of ether in light petroleum in small fractions (2.5 mg. in all) coloured with carotenoids. Ether eluted an orange oil (4.8 mg.), which spectroscopically showed the presence of substance SF ($\lambda_{\max}$ 282 m μ , $E_{1\%}^{1\text{cm}}$ 52) and xanthophylls. The recrystallized sterol showed no selective absorption.

Sheep pancreas. Three samples were studied, carotene, ubiquinone α -tocopherol and substance SF being found in each. The fractions eluted with 4 and 6% of ether in light petroleum were combined, evaporated to dryness under nitrogen and cooled to –25° for 20 min. Methanol (5 ml.) was similarly cooled, poured on to the cold fraction and kept at –25° for 1 hr. The supernatant liquid was decanted and the process repeated (Table 1).

At this stage it was decided to study the lipid as such. Sheep pancreas (259 g.) was homogenized once with ethanol and twice with ether. The combined extracts were washed, dried and the solvent was removed to give 4.1 g. of lipid. A portion of this was chromatographed as lipid and another portion was saponified and the unsaponifiable matter was chromatographed. The extracted tissue was hydrolysed in the presence of pyrogallol. The lipid chromatographed on alumina was eluted to the extent of 40% by light petroleum but total recovery was low (48.4%) because phospholipids were retained. It was not possible even by further chromatography of fractions to concentrate ubiquinone or α -tocopherol successfully. When the

Table 1. Methanol separation of ubiquinone and α -tocopherol at –25°

	$\lambda_{\max}$ (m μ)	$E_{1\%}^{1\text{cm}}$	Wt. (mg.)
Initial product	280	80	12
First methanol-soluble extract	290	54.6	1.6
Second methanol-soluble extract	285–290	33.6	0.6
Third methanol-soluble extract	291	70.9	0.5
Methanol-insoluble	273	14.0	6.8

Table 2. Comparison of pancreatic tissue with embedded lymph-gland tissue constituents of unsaponifiable fraction

	Sheep pancreas		Ox pancreas	
	Pancreatic tissue	Glands	Pancreatic tissue	Glands
Unsaponifiable (%)	0.33–0.37	0.31–0.47	0.41	0.41
α -Tocopherol (mg./100 g.)	0.17	0.05	Not found	Not found
Ubiquinone (mg./100 g.)	0.4–0.6	0.8–1.4	0.88	1.6
Sterol* (g./100 g.)	0.13–0.20	0.15–0.27	0.17	0.29
Formylindole (μ g./g.)	0.7–2.9	?–2.4	1.0–1.2	?–2.1

* 7-Dehydrocholesterol not found.

lipid (2.36 g.) was saponified in the presence of 0.2 g. of pyrogallol with boiling for 10 min. only, 0.2 g. of unsaponifiable matter was obtained. This gave a fraction (1.7 mg.), eluted by 4% of ether in light petroleum, containing both ubiquinone and α -tocopherol, and 6% of ether in light petroleum gave a fraction (3.3 mg.) showing the spectrum of α -tocopherol only. Cholesterol (105 mg.) was eluted by 20% of ether in light petroleum. The unsaponifiable matter from the extracted residue (190 mg.) showed the presence of ubiquinone, α -tocopherol, cholesterol (137 mg.) and substance SF (Table 2).

Pig pancreas. Five small batches of pig pancreas were prepared for analytical purposes and two large batches (17.7 kg.) for preparative purposes. The extracts contained very little carotenoid but ubiquinone, SF and other substances were present. Thus one unsaponifiable fraction (0.77 g.) yielded 14.4 mg. of ubiquinone of 85% purity directly (4–6% of ether in light petroleum eluates), 341 mg. of sterol and 16.4 mg. of a fraction containing substance SF.

Larger-scale preparations were found to contain up to 25% of ethyl palmitate formed by ester exchange. This is easily removed in the first stage of chromatography. In one experiment 3.83 g. of unsaponifiable matter yielded two fractions containing ubiquinone (51 and 22 mg.; $E_{1\text{cm}}^{1\%}$ 117 and 51 respectively). Another fraction contained a congener of m.p. 43°. Ether eluted a fraction containing substance SF.

In brief, ubiquinone was found to the extent of 1–2 mg./100 g. in ox, sheep and pig pancreas. α -Tocopherol (1.6 mg./100 g.) was found only in sheep pancreas. Substance SF was obtained from the three species. The lymph glands also contained ubiquinone and substance SF.

Fat adhering to pancreas was examined separately. Ubiquinone was detected in trace amount in the fat attached to pig pancreas but not in that of sheep or ox. Tocopherol was not found. The fat adhering to ox pancreas yielded unsaponifiable matter which on chromatography revealed the presence of a very small amount of material with λ_{max} 266–267 m μ (cyclohexane) eluted by 5% of ether in light petroleum and a trace of material with λ_{max} 261–262 m μ eluted by 6–7% of ether in light petroleum.

Spleen

Calf spleen. The unsaponifiable fraction contained traces of anhydrovitamin A, β -carotene, ubiquinone and 7-dehydrocholesterol (see Lovern, Morton & Ireland, 1939). Small amounts of substance SF were found in the ether eluate.

Ox spleen. This was similar but somewhat richer in ubiquinone (Table 3). The recrystallized sterol also contained some 7-dehydrocholesterol.

Lamb and ram spleen. Ubiquinone was found (approx. 1 mg./100 g. of tissue) and substance SF, but not α -tocopherol.

Pig spleen. Two batches were studied, from young and old pigs respectively, with results similar to those for other species (Table 3). Chromatography carried out on larger batches resulted in the separation of a congener (m.p. 43°) which follows ubiquinone in the elution and precedes cholesterol.

Human pancreas and spleen

Spleen. The spleens were hydrolysed with ethanolic potassium hydroxide solution in the presence of pyrogallol and prepared in the manner already described. Alumina from the top of the column was boiled with ethanol to yield some

Table 3. *Comparison of spleens of various species: constituents of unsaponifiable matter*

Animal	*Unsaponifiable material (% wet wt.)	Ubiquinone (mg./100 g.)	Sterol (g./100 g.)	7-Dehydrocholesterol (mg./100 g.)	Formylindole (μ g./g.)
Calf	0.445	0.3	0.34	0.06	0.37
Ox	0.44	0.9	0.28	0.02	0.8
Sheep	0.25	0.8	0.27	0.02	0.63
Lamb	0.38	1.1	0.26	0.025	0.47
Ram	0.39	1.0	0.22	0.06	0.45
Pig (old)	0.44	1.9	0.29	0.05	0.5
Pig (young)	0.36	1.2	0.24	0.05	0.5

* α -Tocopherol was not found under these conditions.

Table 4. *Comparison of human spleens: constituents of unsaponifiable matter*

Pathological condition	Sterol* (g./100 g. of tissue)	Ubiquinone (mg./100 g.)	α -Tocopherol† (mg./100 g.)	Formylindole (μ g./g.)
1. Glioblastoma	0.37	2.9	0.24	0.16
2. Carcinoma	0.24	5.9	0.91	1.5
3. Rheumatoid arthritis	0.25	4.3	Not found	1.7

* 7-Dehydrocholesterol was not found in any of these specimens. Cholesta-3:5-dien-7-one was not found. Prelog, Ruzicka & Stein (1943) obtained it from pig spleen.

† Quaife & Dju (1949) found 1.3 mg./100 g. of tissue in the spleen of a normal male aged 30.

substance SF and xanthophylls. The fraction containing ubiquinone and α -tocopherol was left in contact with cold methanol for some hours. The methanol dissolved the α -tocopherol and a clean separation from ubiquinone was effected. The results are shown in Table 4.

Pancreas. Two specimens of pancreas were studied in detail (Table 5). Ubiquinone was present in approximately normal amount but α -tocopherol and 7-dehydrocholesterol were not detected. This exploratory work on human tissue is no more than a beginning and although a positive finding is perhaps significant a negative result may not be so.

Nature of substance SF

Concentrates of substance SF dissolved in *cyclohexane* show absorption peaks at 235 and 280–282 $m\mu$ with an inflexion at 250–255 $m\mu$. In ethanol the peaks are displaced to 242 and 292 $m\mu$ and the inflexion to 260 $m\mu$. The absorption is changed by alkali, and the fact that in spite of this substance SF is extracted from the alkaline hydrolysate of tissue calls for study.

Substance SF is in fact characteristic of the lipid fraction. Thus trimmed sheep pancreas was comminuted in an electric mixer in the presence of acetone. The mixture was centrifuged and the supernatant liquor was poured off. The residue was refluxed with fresh acetone, centrifuged and the process was then repeated. The combined acetone extracts were diluted with water and extracted with ether and the ether extracts were washed and dried and the solvent was removed. This lipid was saponified in the usual way. The protein residue was twice refluxed with ether and centrifuged. The combined ether extracts were washed and the lipid was saponified separately. The protein residue was then refluxed with ethanol, the mixture was centrifuged, the supernatant liquid was removed, reduced to small bulk and saponified. Finally, the protein residue was itself hydrolysed with alkali and the unsaponifiable matter was extracted. Pyrogallol was added in all four saponifications. Each unsaponifiable fraction was partitioned between light petroleum and 80% (v/v) ethanol, 0.02M with respect to sodium ethoxide (the correct weight of sodium metal was dissolved in ethanol and the water added afterwards). The ethanolic layer was examined for selective absorption in the regions around 265 and 325 $m\mu$, a peak at 326 $m\mu$ indicating the presence of 3-formylindole (except in liver tissue, where vitamin A can interfere).

In this experiment 3-formylindole was found only in the unsaponifiable matter derived from the first (acetone) lipid extract. It does not appear from this to be an artifact of tissue protein.

A simple method of testing tissue for 3-formylindole is thus to extract the comminuted material thoroughly with acetone (at least three extractions) and to examine the unsaponifiable fraction of the extracted lipid in the manner described. By this method it may be shown that kidney tissue contains very little formylindole and heart tissue contains none.

If the unsaponifiable matter is dissolved in light petroleum (b.p. 40–60°) and chromatographed on sucrose (icing sugar plus 3% of starch) most of the material is eluted by light petroleum, whereas substance SF is eluted by 2–4% of acetone in light petroleum.

Provisional unit. The working criterion for substance SF is $\lambda_{\max}$, 282 $m\mu$ in *cyclohexane* and the unit used is $E_{1\text{ cm}}^{1\%}$ (282 $m\mu$ /mg.); thus a fraction showing $E_{1\text{ cm}}^{1\%}$ (282 $m\mu$) 15 and weighing 12 mg. would contain 180 units. The amounts found have, however, been expressed in the tables in terms of 3-formylindole.

Partition experiments. It was found to be worth while to dissolve crude SF fractions in a mixture of light petroleum (10 vol.) and absolute ethanol (8 vol.) and then to add water (2 vol.). After shaking for 5 min. the layers were separated and substance SF was markedly concentrated in the ethanol layer. When an SF concentrate ($\lambda_{\max}$, 294 $m\mu$ in ethanol, $E_{1\text{ cm}}^{1\%}$ approx. 85) was distributed between light petroleum and 80% ethanol, made 0.02N with respect to alkali, the light petroleum removed unwanted material and when the ethanol layer was acidified and extracted with ether a material rich in substance SF was obtained, part of it soluble in *cyclohexane* ($\lambda_{\max}$, 282 $m\mu$; $E_{1\text{ cm}}^{1\%}$, 96) and part insoluble ($\lambda_{\max}$, 295, 242 $m\mu$; $E_{1\text{ cm}}^{1\%}$, 231 and 268 respectively).

Micro-high-vacuum distillation of SF fractions obtained by chromatography. The apparatus described by Linstead, Elvidge & Whalley (1955) was used and the vacuum was obtained by means of an oil pump backing a mercury-vapour pump giving 4×10^{-5} mm. Hg. The material used

Table 5. Comparison of human pancreas: unsaponifiable matter

Pathological condition	Unsaponifiable material (g./100 g.)	Sterol (g./100 g.)	Ubiquinone (mg./100 g.)	Formylindole ($\mu\text{g./g.}$)
1. Haemorrhage (Female, 62 years)	0.65	0.23	2.3	Detected
2. Carcinoma (Female, 23 years)	0.76	0.22	2.6	1.75
3. Syphilitic aortitis (Male, 56 years)	0.47	—	—	—

(α -Tocopherol and 7-dehydrocholesterol absent.)

[$E_{1\text{cm}}^{1\%}$ (280 $m\mu$) 45.7, 120 mg.] was from pig spleen. The apparatus was heated by means of a glycerol bath and various fractions were collected. At 125–130° pale-yellow crystals were formed on the cold-finger surface (λ_{max} , 242, 294, 260 $m\mu$; $E_{1\text{cm}}^{1\%}$, 354, 260, 262 respectively). At 145° (60 min.) 6.6 mg. of pale-yellow oil was obtained; it showed the same absorption bands at about one-quarter of the above intensity. Later fractions showed only feeble selective absorption and the residue, a dark-red oil, was of little spectroscopic interest. The first fraction was dissolved in ethanol and taken to dryness in a centrifuge tube. *cyclo*Hexane dissolved part of the material and left some solid behind. The *cyclo*hexane-soluble material was a yellow oil [λ_{max} (ethanol) 240 and 294 $m\mu$; $E_{1\text{cm}}^{1\%}$, 207 and 111]. Redistillation (70°, 4×10^{-5} mm Hg) effected little or no improvement and the product seemed somewhat unstable. The *cyclo*hexane-insoluble fraction showed in ethanol λ_{max} , 242, 260, 295 $m\mu$, $E_{1\text{cm}}^{1\%}$, 755, 650, 773; in ethanolic sodium ethoxide λ_{max} , 266, 326 $m\mu$, $E_{1\text{cm}}^{1\%}$, 1156 and 1095. The fractions distilling at 175–190° showed λ_{max} , 282 $m\mu$, $E_{1\text{cm}}^{1\%}$, 40–60 in ethanolic sodium ethoxide; they may well have contained decomposition products.

Other chromatographic SF fractions were similarly distilled. Finally five distillates (all $E_{1\text{cm}}^{1\%} > 100$) were combined and redistilled. Two fractions were collected at 70°, $E_{1\text{cm}}^{1\%}$ (294 $m\mu$) 222 and 596.

Mass spectra. The mass spectra of the two fractions were kindly obtained for us by Mr A. Quayle of The Thornton Research Laboratory. The fraction with less substance SF showed 'parent' peaks at 392 and 394 with homologues at 406 and 408, although no very strong peak was shown beyond m/e (mass/charge) 177. The purer fraction showed fewer peaks but intense ones occurred at 88, 89, 116, 117, 130, 131 and 144, 145. Small peaks occurred at 256 and 284. The sample contained nitrogen and Mr Quayle considered that the pairs of peaks of similar height were characteristic of nitrogen-containing compounds such as indoles.

Chemical tests. These were carried out on the best distillates. (i) The compound was not oxidized by silver oxide; (ii) it was readily acetylated (acetic anhydride and pyridine) to give a new spectrum; (iii) it gave a silver mirror with ammoniacal silver nitrate. These clues led to indole-aldehyde, and a search of the literature revealed that 3-formylindole very closely resembled the crystalline SF distillate.

The spectra of authentic 3-formylindole in water and in aqueous sodium hydroxide solution were described by Smith (1954) and by Ray & Thimann (1956; in an unstated solvent, probably water). Ray & Curry (1958) described its absorption spectrum in ethanol (see Table 6). Its spectrum in concentrated sulphuric acid is shown in Fig. 1. Fawcett, Taylor,

Wain & Wightman (1958) noted that, in the oxidation by plant tissues of indol-3-yl-acetonitrile, 3-formylindole was formed. They pointed out that it could behave as 3-hydroxy-methylene-indolenine, derivatives of both forms being known:

We have examined authentic 3-formylindole and this isomerism fits the effect of alkali.

Fig. 2 shows good isosbestic points characteristic of a two-component system with the equilibrium almost entirely to one side in ethanol and almost completely to the other side in 0.1 M-alkali.

The extraction of the aldehyde from alkaline solutions by means of ether is a slow process and if the solution is almost neutralized a further yield of 3-formylindole can be obtained.

Other substances isolated. (i) In the course of concentrating substance SF from pig pancreas two other materials were isolated. (a) White crystals

Fig. 1. Absorption curve for 3-formylindole in concentrated sulphuric acid.

(from ethanol), m.p. 131°, no selective absorption of ultraviolet light. Absorption spectrum in conc. sulphuric acid $\lambda_{\max}$. 318 and 416 m μ , $E_{1\%}^{1\text{cm}}$. 96 and 33 respectively. [Found: C, 78.82, 78.75; H, 11.40, 11.49; O, by diff., 9.77 % mol. wt. (Rast) 306, 327. $\text{C}_{22}\text{H}_{38}\text{O}_2$ requires C, 78.98; H, 11.45; O,

Fig. 2. Absorption curves for 3-formylindole. —, In ethanol; ---, in aqueous ethanol, 0.1M with respect to NaOH;, in aqueous ethanol, 5 mm with respect to NaOH; ---, in aqueous ethanol, mm with respect to NaOH. x, Represents a calculated point, assuming a two-component system.

Table 6. Absorption characteristics of 3-formylindole and its N-acetate

Solvent	$\lambda_{\max}$ (m μ)	$\epsilon_{\max}$	$\lambda_{\min}$ (m μ)	$\epsilon_{\min}$
3-Formylindole				
Water	210	27 500	230	6 300
	245	12 600	255	10 700
	262	11 750	277	6 750
	302	13 800	—	—
Ethanol	242	13 700	227	4 700
	260	11 700	252	11 000
	296	13 450	272	7 200
0.02N-NaOH	~250	6 300	235	3 560
	266	17 700	290	6 000
	327	16 000	—	—
Ethanol sodium ethoxide	266	19 900	237	2 500
	326	20 400	293	5 840
Conc. H_2SO_4	235	38 800	220	17 900
	265	54 200	250	28 700
	329	46 700	285	10 100
3-Formylindole N-acetate				
Ethanol	218	31 300	268	6 300
	245	13 600	—	—
	260	6 500	—	—
	298	13 440	—	—

9.57 %; mol.wt. 334]. Liebermann-Burchardt and Zimmermann tests were positive. Infrared absorption in CS_2 3610 (sharp, weak), 2924, 2865, 1745 (sharp, strong), 1377 (~ 1370), 1166 (~ 1155), 1050, 1020, 954, 798 cm^{-1} . Infrared data suggest that the compound is a keto steroid with a hydroxyl group, a carbonyl group in the five-membered ring, but this hypothesis does not fit the analysis. Further work is needed. (b) Pale-yellow crystals were obtained from crude preparations of substance SF from pig pancreas. On recrystallization traces of SF were removed. After five crystallizations the m.p. was 82° (sharp). Unsaturation was indicated by immediate decolorization of a solution of bromine in carbon tetrachloride. The substance readily absorbed oxygen as will be seen from two analyses [Found: C, 77.25, 76.98; H, 12.9, 12.3; O, by diff., 9.84, 10.72; mol.wt. 511 Rast. A later analysis gave C, 75.58; H, 12.28; O, by diff., 12.14 %, and later still C, 72.21; H, 12.23; O, by diff., 15.56; mol.wt. (Rast) 516. $\text{C}_{33}\text{H}_{64}\text{O}_3$ requires C, 77.88; H, 12.68; O, 9.46 %, $\text{C}_{33}\text{H}_{64}\text{O}_4$ requires C, 75.5; H, 12.30; O, 12.20 %, $\text{C}_{33}\text{H}_{64}\text{O}_5$ requires C, 73.33; H, 11.95; O, 14.80 %]. After further recrystallization in the presence of animal charcoal the m.p. rose to 86.5°. The infrared absorption showed a strong sharp band due to hydroxyl at 3332 cm^{-1} in liquid paraffin and 3460 cm^{-1} in chloroform. Other strong bands occurred at 2915, 2857, 1642, 1613, 1563, 1543, 1462, 1372 and 716 cm^{-1} . The compound probably contains ethylenic linkages, and it may be an unsaturated glycerol ether, $\text{C}_{30}\text{H}_{57}\cdot\text{O}\cdot\text{CH}_2\cdot\text{CH}(\text{OH})\cdot\text{CH}_2\cdot\text{OH}$.

(ii) A third compound from pig pancreas was obtained from ubiquinone fractions. From its infrared spectrum it was an aliphatic ketone or aldehyde (1736, 2915, 2849, 1468, 1464, 1193, 1171, 1148, 1107, 1094, 718 cm^{-1}). The compound showed weak selective absorption at 270 $\text{m}\mu$; m.p. 43°.

Further work is needed on all three substances. They are only incidental to the present study.

DISCUSSION

Ubiquinone at the concentration of 1–2 mg./100 g. of tissue occurred in the pancreas of ox, sheep, pig and man. It was also found in lymph glands embedded in the pancreatic tissue and it was also present in spleen. α -Tocopherol, found in sheep pancreas, was either absent from pancreas of ox and pig or present in much lower concentration. Tocopherol was not detected in animal spleen but was found in human spleen. Cholesterol obtained from spleen contained detectable amounts of 7-dehydrocholesterol (0.02–0.06 mg./100 g.), but this substance was lacking in the sterol from pancreas.

The amount of ubiquinone found in human spleen may possibly exceed that found in animals,

but more tests are needed. Similarly, the absence of 7-dehydrocholesterol needs to be confirmed on more specimens.

The detection of 3-formylindole is interesting. Salhanick & Berliner (1957) studied a large feminizing adrenal carcinoma from a man aged 22, from which they isolated progesterone, equilenin and five unidentified materials, two of which were designated AAE-I-AI and AAE-I-BI. Salhanick & Berliner (1957) recorded for AAE-I-AI absorption peaks at 242, 260 and 295 $\text{m}\mu$ in methanol and at 240, 265 and 330 $\text{m}\mu$ in concentrated sulphuric acid. Their published curves display maxima and minima with clear patterns of relative intensities of absorption. The total amount of material available (132 $\mu\text{g}.$) was very small and there was no evidence about purity. The preparation gave a positive Millon's test (thought to indicate a 3:5-unsubstituted hydroxyphenyl group) and a positive Zimmermann test for 17-oxo steroid. Both tests are now seen to be due to congeners and not to the selectively absorbing entity. There can be no doubt that the latter was indistinguishable from 3-formylindole. This compound shows three maxima and minima in each of two very different solvents and also reproduces the patterns of intensity seen in the diagrams of Salhanick & Berliner (1957).

Ray & Curry (1958) studied the photochemical destruction of indolylacetic acid by monochromatic light (280 $\text{m}\mu$) and found 3-formylindole among the products. They quoted Stutz as having observed that enzymic reduction, which does not normally lead to 3-formylindole, does so when cytochrome *c* and a cytochrome oxidase are present in addition to indolylacetic acid oxidase.

As already noted, Fawcett *et al.* (1958) found that plant tissues could form 3-formylindole from indol-3-yl-acetonitrile. Jones & Taylor (1957) detected in cabbage the acetonitrile, indole-3-carboxylic acid as well as 3-formylindole.

The question whether 3-formylindole is as such biologically significant in animal tissues cannot yet be answered. That it is an artifact is very possible. It is not, however, found in all tissues, which may indicate that it is not a normal artifact from the tryptophan of tissue protein. The small yield suggests that if 3-formylindole is an artifact it must arise from a highly specific precursor. This seems to be a good working hypothesis.

SUMMARY

1. Pancreas and spleen from ox, sheep and pig have been found to contain small amounts of ubiquinone. This substance has also been found in human spleen and pancreas. In the course of the work the distribution of α -tocopherol and 7-dehydrocholesterol have been studied.

2. Unaponifiable fractions from spleen and pancreas contain very small amounts of a substance spectroscopically identical with 3-formylindole.

3. The relationship of the absorption spectra in two solvents of a fraction isolated by Salhanick & Berliner (1957) from a suprarenal tumour to those of 3-formylindole is discussed.

We wish to express our gratitude to the Nuffield Foundation for a grant towards the cost of investigation. We are indebted to the Thornton Research Laboratory for mass spectra, to Professor H. L. Sheehan for post-mortem material and to Dr J. B. Jepson for 3-formylindole.

REFERENCES

- Fawcett, C. H., Taylor, H. F., Wain, R. L. & Wightman, F. (1958). *Proc. Roy. Soc. B*, **148**, 543.
- Festenstein, G. N., Heaton, F. W., Lowe, J. S. & Morton, R. A. (1955). *Biochem. J.* **59**, 558.
- Heaton, F. W., Lowe, J. S. & Morton, R. A. (1955). *Biochem. J.* **60**, xviii.
- Heaton, F. W., Lowe, J. S. & Morton, R. A. (1957). *Biochem. J.* **67**, 208.
- Jones, E. R. H. & Taylor, W. C. (1957). *Nature, Lond.*, **179**, 1138.
- Linstead, R. P., Elvidge, J. A. & Whalley, M. (1955). *A Course in Modern Techniques of Organic Chemistry*, p. 51. London: Butterworths Scientific Publications.
- Lovern, J. A., Morton, R. A. & Ireland, J. A. (1939). *Biochem. J.* **33**, 325.
- Morton, R. A., Wilson, G. M., Lowe, J. S. & Leat, W. M. F. (1957). *Chem. & Ind.* p. 1649.
- Prelog, V., Ruzicka, L. & Stein, P. (1943). *Helv. chim. acta*, **26**, 2222.
- Quaife, M. & Dju, M. Y. (1949). *J. biol. Chem.* **180**, 263.
- Ray, P. M. & Curry, G. M. (1958). *Nature, Lond.*, **181**, 895.
- Ray, P. M. & Thimann, K. V. (1956). *Arch. Biochem. Biophys.* **64**, 175.
- Salhanick, H. A. & Berliner, D. L. (1957). *J. biol. Chem.* **227**, 583.
- Smith, G. F. (1954). *J. chem. Soc.* p. 3842.
- Tosic, J. & Moore, T. (1945). *Biochem. J.* **39**, 498.
- Williams, T. I. (1948). *An Introduction to Chromatography*, p. 13. London: Blackie and Son. Based on Brockmann, H. & Schodder, H. (1941). *Chem. Ber.* **74**, 73.

Unaponifiable Fraction of Lipid from Normal and Diseased Human Kidney

BY L. MERVYN AND R. A. MORTON

Biochemistry Department, The University of Liverpool, Liverpool 3

(Received 27 August 1958)

Lowe, Morton & Vernon (1957) reported on the unaponifiable constituents of kidneys from a number of species. Vitamin A is normally present, but in amounts so small that the fractionation of kidney unaponifiable matter is not hindered by the presence of artifacts derived from vitamin A. The two substances SA and SC (Heaton, Lowe & Morton, 1955, 1957) were present in kidneys, the amounts being of the order 20 $\mu\text{g./g.}$ for SA and 5 $\mu\text{g./g.}$ for SC, on the assumption that the best preparations so far obtained are not far from pure. Vitamin E was detected in the kidneys of dog, sheep and cat. It was found that the vitamin A content of cat kidney was very much greater than that of kidney from other species; similarly, the vitamin E content was quite unusually high. In the cat kidney vitamins A and E were found predominantly in the cortex and vitamin E was not detected in the medulla.

Much of the material used by Lowe *et al.* (1957) was from healthy animals which had been killed only a very short time before the kidney tissue was

saponified. Post-mortem changes were therefore usually small and often minimal.

The study of human kidney tissue offers both advantages and disadvantages. On the debit side is the fact that the kidney tissue which becomes available through the co-operation of pathologists has often had time to undergo change, and moreover usually comes from a person who had died of disease rather than accident. Among the advantages of studying human tissue is the fact that very full medical records may often be consulted so that any abnormalities in the chemical constituents of kidney tissues can be considered in the light of clinical and pathological observations.

The present investigation is largely exploratory. The structures of substances SA and SC, as well as their biological significance, are the subjects of parallel research. The name ubiquinone has been given to substance SA because it has been shown to be very widely distributed and to occur at sites which imply that it exhibits catalytic properties.