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Unsaponifiable Constituents of Liver; Ubiquinone and Substance SC in Various Species

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The determination of substances present in very small amounts in the unsaponifiable fraction of liver lipids is made difficult by the fact that complete hydrolysis requires the tissue or lipid to be exposed to strong alkali on the boiling-water bath. Substances such as vitamins K and E are alkali-labile and indeed may be destroyed entirely. Cain & Morton (1955) found that when vitamin A was a relatively plentiful component of the liver, chromatographic and other methods of separation were often vitiated by the presence in the unsaponifiable fraction of vitamin A-decomposition products.

The substance SA of Festenstein, Heaton, Lowe & Morton (1955) and Cain & Morton (1955) was also encountered by Lowe, Morton & Harrison (1953) and by Heaton, Lowe & Morton (1955, 1957). Because of its widespread occurrence SA has been designated ubiquinone (Morton, Wilson, Lowe & Leat, 1957).

A second minor constituent of liver unsaponifiable material designated substance SC, was found in very small amounts in normal rat liver (Heaton *et al.* 1955) and larger amounts of both substance SC and ubiquinone were found in liver from vitamin A-deficient rats.

The main object of the present investigation was

to determine whether ubiquinone and substance SC occur in the normal livers of various species.

Festenstein *et al.* (1955) found ubiquinone to be present in various tissues of pig, horse and rat, including normal rat liver. They showed that it was not a saponification artifact, since the same amount was present in the total lipid as in the unsaponifiable material from the tissue. Saponification of the extracted lipid, however, destroyed it. Thus since it is labile to alkali, ubiquinone is evidently protected in some way during the alkali digestion of the tissue. Ward & Moore (1955) confirmed the occurrence of an alkali-labile substance with the absorption band at 272 m μ of SA in the unsaponifiable material from vitamin A-deficient rat liver. They also observed similar spectra in liver fractions (a) from rats cured of vitamin A deficiency, (b) from sexually immature rats and (c) from normal pigs and normal guinea pigs. Cain & Morton (1955), in an extensive investigation of horse-liver unsaponifiable matter, found ubiquinone, usually accompanied by cholesta-3:5-dien-7-one. Although both substances show absorption peaks near 270 m μ , ubiquinone can readily be distinguished from the dienone. The latter is stable in alkaline solution and its spectrum in concentrated sulphuric acid has a characteristic peak at 356 m μ ; ubiquinone-

containing fractions, on the other hand, show no maximum at 356 $m\mu$ in concentrated sulphuric acid, but usually have a band with $\lambda_{\max}$ at 315 $m\mu$ (Kantiengar, Lowe, Morton & Pitt, 1955).

EXPERIMENTAL

Materials

Livers from healthy oxen, sheep and pigs were obtained from the Stanley Abattoir, Liverpool, and were always quite fresh. Cat livers were obtained from animals killed by the R.S.P.C.A. at the request of the owners. Dog livers were obtained from the R.S.P.C.A. or the Liverpool University Veterinary Hospital. Guinea-pig livers were obtained from animals kept in the Departmental Animal House for several months on a diet of cubes (Animal Diet no. 4, British Extracting Co., Bromborough, Cheshire) and fresh cabbage. Horse liver was obtained from Lasco Ltd., Liverpool, very soon after slaughter. The livers of 50 mice were obtained from Burroughs Wellcome and Co., London, and were examined as one lot; a similar batch of 50 livers was obtained from The Distillers Co. Ltd. (Speke). Livers from a number of pigs suspected of being vitamin A deficient were obtained from the Veterinary Investigation Centre, Liverpool, through the courtesy of Mr N. H. Brooksbank. The livers of three pullets were obtained from J. Bibby and Sons Ltd., Hann's Hall Farm, Willaston, Cheshire. Rabbit livers came from laboratory animals on a cube and cabbage diet. Human liver was provided by the Department of Pathology, University of Liverpool, through the courtesy of Professor H. L. Sheehan.

Methods

Saponification. If the liver could not be prepared very soon after reaching the laboratory it was stored at -20° , but this was usually not necessary. The tissue was cut into small pieces and refluxed on a water bath for 45–60 min. with a mixture of 60% (w/v) potassium hydroxide (0.5 ml./g. of tissue) and ethanol (1–2 ml./g. of tissue) containing pyrogallol (0.25%, w/v) which was added just before mixing. After cooling, water (2 vol.) was added and the solution extracted with five or six portions of ether (previously redistilled over reduced iron). The extract was washed, dried over Na_2SO_4 , filtered through sintered glass and the solvent removed in a stream of nitrogen. The last traces of solvent were removed under reduced pressure in a 25 ml. flask on the water bath.

Chromatography. This was carried out on alumina (P. Spence and Co., Widnes) partially deactivated with water to Brockman Grade 3 (Williams, 1948). The column was washed with light petroleum (100–200 ml.), b.p. 40–60°, before use. The unsaponifiable matter was poured on to the column in light petroleum solution and the chromatogram was developed, at first with the pure solvent and then with mixed solvents consisting of light petroleum (b.p. 40–60°) containing increasing percentages of diethyl ether. The more polar compounds were finally eluted with ether and in some cases ethanol. All fractions were evaporated to dryness under nitrogen and weighed before spectroscopic examination in cyclohexane. They were stored at -20° in the dry state in small conical flasks.

Spectroscopy. The weakly adsorbed material (mainly hydrocarbon) eluted from the alumina column by light petroleum showed an inflexion at 260 $m\mu$ (in cyclohexane)

but was otherwise free from selective absorption. α -Tocopherol and 7-dehydrocholesterol were determined by means of their well-known absorption spectra. Ubiquinone shows (in cyclohexane) $\lambda_{\max}$ 272 $m\mu$ ($E_{1\text{cm}}^{1\%}$ taken as 180 in calculating $\mu\text{g./g.}$) with an inflexion at 330 $m\mu$ and a weak band at 410 $m\mu$. Substance SC (in cyclohexane) shows two peaks, at 275 and 330 $m\mu$ respectively, with marked inflexions at 233 and 283 $m\mu$ (Fig. 1). Since the molecular extinction coefficients of SC are not known, the SC content of a preparation is expressed in arbitrary units xy , where $E_{1\text{cm}}^{1\%}$ 275 $m\mu$ is measured (x) and the weight of the fraction is given in mg. (y).

Substance SC may occur generally in healthy liver but in most species the amount is so very small that no chromatographic fraction shows the characteristic absorption spectrum unambiguously. It is here reported as not detected (n.d.) unless the full spectrum was obtained, even though some fractions may have contained traces of substance SC but exhibited distorted spectra.

Preliminary separation of unsaponifiable fraction. Many samples of mammalian or avian liver contain up to 3000 i.u. of vitamin A/g., and this can with advantage be largely eliminated before undertaking chromatography. Cholesterol is readily crystallized from methanol and vitamin A remains in solution; ubiquinone and substance SC tend to accompany the cholesterol (Fig. 2). The first crop of cholesterol obtained in this way from a sheep-liver unsaponifiable fraction showed $\lambda_{\max}$ 275 $m\mu$ due mainly to ubiquinone.

A methanolic solution of dog-liver unsaponifiable matter was cooled in solid carbon dioxide and the methanol-insoluble fraction was quickly filtered off. The filtrate was warmed and cholesterol (0.2 g.) was added and dissolved. The solution was again cooled strongly and filtered. The

Fig. 1. —, Absorption curve of ubiquinone in cyclohexane; 1 cm., 0.006%. ---, Absorption curve of substance SC in cyclohexane; 1 cm., 0.0054%.

Fig. 2. Absorption curves of unsaponifiable matter of sheep liver. ---, Methanol-soluble fraction in cyclohexane (1 cm., 0.00176 %); —, methanol-insoluble fraction in cyclohexane (1 cm., 0.617 %).

Fig. 3. Absorption curves of unsaponifiable matter of dog liver. 1 (—), Methanol-soluble fraction in cyclohexane (1 cm., 0.0172 %); 2 (---), methanol-insoluble fraction in cyclohexane (1 cm., 0.307 %); 3 (- · - ·), sterol obtained after adding cholesterol to 1 and recrystallizing from methanol; insoluble fraction in cyclohexane (1 cm., 0.48 %); 4 (....), cholesterol added to mother liquor from 3 and crystallized. Methanol-insoluble portion in cyclohexane (1 cm., 0.270 %).

second crop of cholesterol crystals carried down an additional amount of ubiquinone. Addition of further cholesterol (0.2 g.) did not result in any further appreciable yield of material absorbing at 270–275 $m\mu$ (Fig. 3).

This procedure allowed most of the vitamin A to be separated from the mixture of cholesterol and ubiquinone. A careful test on a sample of unsaponifiable matter from normal pig liver, containing only a moderate amount of vitamin A, showed that the yield of ubiquinone obtained by chromatography of the whole unsaponifiable fraction was about 30 $\mu\text{g./g.}$; of this about 22 $\mu\text{g./g.}$ was readily carried down by cholesterol. On the alumina column, ubiquinone is readily separable from cholesterol, but substance SC and 7-dehydrocholesterol tend to be eluted with cholesterol.

Acetylation of sterol fraction and rechromatography. The main sterol fraction eluted by 20 % (v/v) ether in light petroleum can with advantage be acetylated (acetic anhydride and pyridine) and rechromatographed. Under these circumstances cholesteryl acetate, vitamin A acetate and 7-dehydrocholesteryl acetate can all be eluted by 1 % (v/v) ether in light petroleum and any small amounts of more polar products can be eluted later with more polar mixtures of the two solvents.

RESULTS

The findings for the different species are first described and then summarized in Table 6.

Cat. The two cats studied gave liver unsaponifiable fractions exhibiting strong vitamin A absorption. Most of the vitamin A remained in solution after crystallization of cholesterol from methanol as described earlier. Cholesterol was added to the mother liquor and a fresh crop of crystals obtained. Table 1 shows that carotene and lycopene were detected along with 7-dehydrocholesterol, ubiquinone and two other entities, SB and SF (Morton & Fahmy, 1959). Rechromatography of the acetylated sterol fraction failed to reveal the presence in it of any substance SC.

Dog. Ubiquinone was present in fractions eluted by 4 % (v/v) ether in light petroleum and 6 % (v/v) ether in light petroleum. The main sterol fraction from dog 1 was acetylated and rechromatographed. The portion (0.7 mg.) eluted by 8 % ether in light petroleum showed an inflexion at 227 $m\mu$, a peak at 275 $m\mu$ ($E_{1\text{cm.}}^{1\%}$ 11.0) with inflexions at 282 and 315 $m\mu$, all consistent with the presence of a trace of substance SC. The liver of the other dog did not give a detectable amount of SC.

Guinea pig. Four livers (103 g.) gave 0.311 g. of unsaponifiable matter showing maxima at 273, 282 ($E_{1\text{cm.}}^{1\%}$ 3.8 and 5.1 respectively) and 328 $m\mu$. The vitamin A content was quite low (26 i.u./g. of liver), and it was therefore convenient to chromatograph the whole unsaponifiable fraction. The ubiquinone fraction was contaminated with a substance showing $\lambda_{\text{max.}}$ 297 $m\mu$ which turned out to be α -tocopherol. The next fraction also showed the tocopherol spectrum superimposed on that of

substance SC. The sterol fraction contained 7-dehydrocholesterol. After the sterol had been eluted, ether removed SF ($\lambda_{\max}$ 236, 282 $m\mu$; $E_{1\text{cm}}^{1\%}$ 48.0 and 36.3). Ethanol eluted an appreciable amount of xanthophyll pigment. Rechromatography of the acetylated sterols confirmed the presence of 7-dehydrocholesterol and the absence of substance SC from the sterol fraction.

Horse. The two horse livers were low in vitamin A. Ubiquinone was present in both but neither substance SC nor SF could be detected unambiguously.

Mouse. The two specimens of mouse liver differed widely in vitamin A content. Nevertheless, ubiquinone was present in both at about the same rather high proportion (Table 2). The separation (Table 2) was clear, partly because of the absence of complicating carotenoids. Substance SC was not separated but SF was probably present in small amount.

Ox. Three samples were examined; the ubiquinone content was variable but substance SC was not detected although a distorted SC spectrum was suspected in one fraction.

Table 1. *Chromatography of methanol-insoluble portion of cat-liver unsaponifiable fraction*

Wt. of liver 129 g.; crude unsaponifiable matter 0.892 g.; cholesterol added to unsaponifiable matter 0.200 g. Methanol insoluble material 0.585 g.; a 200 mg. portion of this was chromatographed. Alumina column 20 g. E, Ether; P, light petroleum.

Fraction no.	Eluent (ml.)	Wt. (mg.)	Absorption spectra in cyclohexane	
			$\lambda_{\max}$ ($m\mu$)	$E_{1\text{cm}}^{1\%}$
1	200 P + 50 (1%) E/P	3.5	230	67.5
			260	31.6
			333	17.3
			432	42.8
			451	53.1
			480	43.7
2	200 (2%) E/P	1.2	260	21.1
			450	6.5
			Max. 472	6.7
			495	4.2, lycopene
3	200 (4%) E/P	0.9	Max. 272	81.7, ubiquinone
			330	14.4
			362	9.3
			Max. 445	9.6
			Max. 472	10.1, lycopene
			Max. 505	6.9
4	200 (6%) E/P	8.4	Max. 274	3.2
			325	0.8
5	100 (8%) E/P	1.7	No selective absorption, contains cholesterol	
6	700 (20%) E/P	181.8	257	0.68
			265	0.70, 7-dehydrocholesterol
			275	0.71
			285	0.62
			Max. 325	0.50, vitamin A
7	200 (E)	1.3	Max. 287	19.0, ? SF

(b) Fraction 6: acetylated and rechromatographed
(0.1975 g. was placed on 20 g. column)

Fraction no.	Eluent (ml.)	Wt. (mg.)	Absorption spectrum in cyclohexane	
			$\lambda_{\max}$ ($m\mu$)	$E_{1\text{cm}}^{1\%}$
1	400 (1%) E/P	191.4	7-Dehydrocholesterol and vitamin A acetate	
2	300 (4%) E/P	1.1	235	0.81
			270	0.40
3	300 (8%) E/P	0.4	235	4.86
			275	2.43
4	200 E	Trace	233	?
			270	—

Table 2. *Chromatography of mouse-liver unsaponifiable fraction*

Wt. of liver 88 g. (50 mice); unsaponifiable matter 0.277 g.; vitamin A, 10 i.u./g. of liver; unsaponifiable matter (0.258 g.) chromatographed on 20 g. of alumina. A second batch of mouse liver (from another colony) contained 151 i.u. of vitamin A/g., but the ubiquinone content was nearly the same (see Table 6). E, Ether; P, light petroleum.

Fraction no.	Eluent (ml.)	Wt. (mg.)	Absorption spectrum in cyclohexane	
			$\lambda_{\max}$ (m μ)	$E_{1\%}^{1\text{cm}}$
1	200, P	7.3	257	11.1
			325	1.6
2	200 (2%) E/P	0.2	280	51.5
3	300 (4%) E/P	4.2	Max. 273	140.4
			320	14.4
4	200 (6%) E/P	3.1	Min. 248	13.5, mainly ubiquinone
			Max. 276	24.9
			320	3.4
5	100 (8%) E/P	1.8	Max. 276	7.9, SB
			310	4.6
6	800 (20%) E/P	161	235	1.4
			287	1.3
			320	1.9
			Max. 327	2.0, vitamin A
			232	192
7	200 E	0.3	252	148
			Max. 280	141
			292	119
				SF

Table 3. *Constituents of the unsaponifiable fractions of livers of pigs which died at 4-5 months of age*

—, Not measured. Substance SC was detected in trace amounts in samples 1, 2 and 4.

Sample no.	Wt. of liver	Unsaponifiable matter as percentage of liver weight	Total sterol (mg./g. of liver)	Vitamin A (i.u./g. of liver)	$\lambda_{\max}$ (m μ)	$E_{1\%}^{1\text{cm}}$	Ubiquinone ($\mu\text{g./g. of liver}$)	7-Dehydro-cholesterol ($\mu\text{g./g. of liver}$)
1	376	0.40	2.5	2.2	272	3.9	34.6	Not seen
2	173	0.41	2.3	4.2	272	3.4	46.8	Not seen
3	95	0.44	—	3.8	273	4.6	—	—
4	240	0.37	2.2	None	272	3.6	19.3	0.5

Pig. Three lots of liver from healthy pigs were examined. The vitamin A contents varied from 6 to 150 i.u./g. of liver but the ubiquinone varied only from 9 to 30 $\mu\text{g./g.}$ Substance SB was present but SC could not be detected. Four samples were studied of liver from pigs which had died at the age of 4-5 months with symptoms of inco-ordination of the hind legs and increased excitability (Table 3). Although the ubiquinone content was rather high it was not at all clear that in pig liver this substance or SC increases in vitamin A deficiency. Substance SC was detected in small amounts and SF was present in the ethanol eluate from the alumina.

Pullet. The liver of the normal hen is a source of substance SC. Table 4 illustrates the chromatography of the methanol-insoluble unsaponifiable fraction. Fraction 5 (Table 4) was acetylated and rechromatographed. Fig. 4 illustrates the ab-

sorption spectrum of the 8% of ether in light petroleum eluate, which agrees with that of substance SC.

Rabbit. Two livers (104 g.) were bulked and the vitamin A content was determined (617 i.u./g.); cholesterol (0.2 g.) was added to the total unsaponifiable material in methanol. The crystallized cholesterol carried down ubiquinone but little vitamin A. The complete SC spectrum was not seen, but a trace of SC may have been present.

Sheep. The first two of the specimens studied (Table 6) were early ones, before the methanol crystallization with added cholesterol was adopted, and neither ubiquinone nor substance SC could be detected in the presence of vitamin A. In sheep 3 and 4, normal amounts of ubiquinone and minute traces of SC were detected.

Man. Table 5 records the results of examination of three human livers. Ubiquinone was present in

Table 4. *Chromatography of methanol-insoluble fraction of pullet-liver unsaponifiable matter*

Wt. of liver 124 g.; unsaponifiable matter 0.57 g.; vitamin A 950 i.u./g. of liver; cholesterol (0.2 g.) was added to 0.56 g. of unsaponifiable material dissolved in methanol and cooled. The crystalline product (0.55 g.) showed λ_{max} 273 m μ ; $E_{1\text{cm}}^{1\%}$ 3.4; 0.2 g. of this was chromatographed. E, Ether; P, light petroleum.

Fraction no.	Eluent (ml.)	Wt. (mg.)	Absorption spectrum in cyclohexane	
			λ_{max} (m μ)	$E_{1\text{cm}}^{1\%}$
1	50 (1%) E/P + 150 (2%) E/P	— 1.2	Max. 260	19
			Max. 331	12
			454	26, carotene
			482	22
2	300 (4%) E/P	1.9	Max. 272	150, ubiquinone
			320	14
3	200 (6%) E/P	3.0	Max. 272	26, ubiquinone
			320	2.8
4	100 (8%) E/P	1.1	Max. 274	73, SC?
			320	18.7
5	1000 (20%) E/P	176	Max. 274	0.41
			280	0.40
			320	0.31
			Max. 328	0.32, vitamin A
6	200 E	0.4	230	40
			270	26

DISCUSSION

The unsaponifiable material of liver dissolved in methanol and strongly cooled deposits crystals of cholesterol, accompanied by ubiquinone, substance SC and 7-dehydrocholesterol. Vitamin A remains in the mother liquor and by adding further cholesterol and again crystallizing, additional amounts of trace constituents can be carried down. Chromatography then allows ubiquinone to be separated but SC remains with the sterol. If the sterol is acetylated, SC can be separated by chromatography from cholesteryl acetate. These procedures may lead to estimates erring on the low side because of losses occurring in the saponification. The use of pyrogallol in the hydrolysis greatly reduces such losses.

In the chromatographic separations 2–4 % of ether in light petroleum eluted (well ahead of the cholesterol) a small amount of yellow oily material. This fraction was rich in ubiquinone. It was followed, with 4–6 % of ether in light petroleum, by a substance in which λ_{max} was shifted from 272 to 274 m μ (solvent cyclohexane) and the inflexion at 330 m μ was more marked than for ubiquinone. This substance, called SB for convenience, either possesses very low intensity of absorption or is in these experiments much diluted with congeners transparent to ultraviolet light. Less strongly adsorbed than ubiquinone is a hydrocarbon fraction eluted by petrol or 1 % of ether in light petroleum. This almost always contains a substance showing an inflexion near 233 m μ and a small maximum or inflexion near 260 m μ .

Fig. 4. Absorption curves in cyclohexane of unsaponifiable fraction containing substance SC acetate from hen liver. This fraction was obtained by rechromatography of a fraction containing cholesterol and substance SC which had been acetylated to facilitate separation. —, 0.0267%; ---, 0.0134% (both measured in 1 cm. cells).

all, but substances SB, SC and SF could not be detected. Table 6 illustrates another case.

Table 6 recalls the main findings and Table 7 shows how widely the vitamin A content can vary compared with that of ubiquinone.

Table 5. *Constituents of the unsaponifiable fraction from human livers*

Carotene and lycopene were present in traces in all three livers; tocopherol was found only in the third and substance SC was not detected in any of the three. These three specimens were examined by Dr L. Mervyn.

Sex	Age (years)	Cause of death	Wt. of liver (g.)	Percentage of unsaponifiable material	Sterol (mg./g. of liver)	Vitamin A (i.u./g.)	Ubiquinone (μ g./g. of liver)
F.	72	Internal haemorrhage	120	0.74	4.8	2000	53
F.	20	Acute nephritis	110	0.68	3.2	240	56
M.	43	Uncertain	150	0.49	2.7	132	75

Table 6. *Unsaponifiable constituents of liver*

n.d., Not detected; d, detected; a, carotene; b, lycopene; c, xanthophylls; e, 7-dehydrocholesterol.

Species	Sterol (mg./g. of liver)	Vitamin A (i.u./g. of liver)	Ubiquinone (μ g./g.)	Substance SC	Other compounds detected
Cat (i)	2.4	380	9	n.d.	a, b, e
(ii)	2.6	2320	13	n.d.	a, b, e
Dog (i)	1.3	104	20	d.	e
(ii)	1.4	61	3	n.d.	—
Guinea pig	2.0	26	11.6	?	c, e
Horse (i)	1.2	46	12	n.d.	e
(ii)	1.4	21	6	n.d.	a, e
Man	2.1	2000	12.7	n.d.	a, b
Mouse (i)	1.9	151	45	n.d.	e
(ii)	1.9	10	41	n.d.	e
Ox (i)	1.7	215	35	n.d.	a
(ii)	1.9	87	11.5	n.d.	a, b, e
	1.7	380	3	?	a, e
Pig (i)	2.4	6	9	n.d.	a
(ii)	1.7	15	24	n.d.	e
(iii)	3.1	160	30	n.d.	—
Pullet (i)	4.5	950	108	0.8 unit/g.	a, e
(ii)	2.4	1060	46	0.4	a, e
Rabbit	1.9	617	12.7	?	e
Sheep (i)	3.7	960	—	—	a
(ii)	3.1	540	—	—	a
(iii)	2.2	1830	15	d.	a, e
(iv)	3.8	1270	33	d.	a, e

Table 7. *Comparison of liver content of ubiquinone and vitamin A*

	Vitamin A (i.u./g.)	Ubiquinone (μ g./g.)
Cat	382	8.5
	2320	12.5
Pig*	15	24.5
	160	29.8
Mouse	10	41
	151	44

* See also Table 3.

Substance SC, eluted by 20% of ether in light petroleum ($\lambda_{\max}$, 275, 330 m μ , inflexions 233, 283 m μ in cyclohexane) was unmistakably observed in dog, sheep and pullet liver. It was not definitely detected in pig and other livers even if

the vitamin A content was less than 10 i.u./g. of liver. In the rat, the SC content rises significantly in vitamin A deficiency (Heaton *et al.* 1955).

SF ($\lambda_{\max}$, 282 m μ) is occasionally found in liver unsaponifiable matter. It is very strongly held on alumina. This substance has been shown (Morton & Fahmy, 1959) to be 3-formylindole or a compound with the same absorption characteristics.

SUMMARY

1. Liver tissue saponified in the presence of pyrogallol yields unsaponifiable matter of which ubiquinone is a normal constituent.

2. The cholesterol which crystallizes from a methanolic solution of the unsaponifiable matter carries with it ubiquinone and substance SC. Addition of cholesterol to the mother liquor leads

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.

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

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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.

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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.