2. Unsaponifiable 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.

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Unsaponifiable Fraction of Lipid from Normal and Diseased Human Kidney

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Lowe, Morton & Vernon (1957) reported on the unsaponifiable constituents of kidneys from a number of species. Vitamin A is normally present, but in amounts so small that the fractionation of kidney unsaponifiable 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.

It has a high oxidation-reduction potential and behaves like a typical quinone (Morton, Wilson, Lowe & Leat, 1957).

MATERIALS AND METHODS

Human kidneys were obtained through the help of Professor H. L. Sheehan, Dr A. H. Cruickshank and their colleagues in the Department of Pathology, University of Liverpool. In addition, kidneys from patients who had died from nephritis were obtained from Dr J. C. Brundret of Sefton General Hospital, Liverpool, Dr F. B. Mooney of St Helens' Hospital, Dr R. M. Rawcliffe of St Catherine's Hospital, Birkenhead, Dr R. E. Newell of the Royal Liverpool Children's Hospital and Dr M. J. J. Wodzinski of Walton Hospital, Liverpool. Human blood was obtained through the aid of Dr D. Lehan of the Regional Blood Transfusion Service.

As soon as kidneys were received, the perinephric fat and membrane were removed, together with most of the pyramid. Each kidney was cut longitudinally and the halves were washed in water, dried roughly on filter paper and weighed. If it was not possible to proceed at once the tissue was covered with ethanol and left at -20° .

Saponification. The tissue was cut into small pieces and digested on the water bath with 60% (w/v) KOH (0.5 ml./g. of tissue) until the mixture became homogeneous; ethanol (1 ml./g. of tissue) was then added and the mixture boiled under reflux for 30–45 min. It was later found that it is better to mix the 60% KOH and ethanol (with dissolved pyrogallol) before bringing the tissue into contact with alkali; this minimizes loss of vitamin E. After cooling, water (2 vol.) was added and the solution was then extracted six times with ether (dried over sodium and then freshly distilled over reduced iron). The combined ether extracts were washed free from alkali, dried over anhydrous Na_2SO_4 , filtered through a sintered-glass funnel, evaporated to dryness under nitrogen and weighed.

Determination of vitamin A. This was carried out on small portions of the unsaponifiable matter (30–40 mg.) with the antimony trichloride reaction (Glover, Goodwin & Morton, 1947).

Chromatography. The unsaponifiable matter was chromatographed on alumina (Brockmann grade 3), as described in previous papers (Heaton *et al.* 1957; Lowe *et al.* 1957). Grade 0 alumina (P. Spence and Co., Widnes) was partially deactivated with water to Brockmann grade 3, and as a rule 10 g. was taken for each 100 mg. of unsaponifiable matter. The procedure was to dissolve the material in light petroleum (b.p. $40-60^{\circ}$) and to develop the chromatogram with the same solvent. At this stage a small amount of hydrocarbon (inflexions at 235 and 260 $\text{m}\mu$) was eluted. The chromatogram was further developed with 2% (v/v) of ether in light petroleum, which eluted any remaining aliphatic hydrocarbons, lycopene, carotene and probably any cholesta-3:5-dien-7-one. Ubiquinone was eluted by means of 4% (v/v) of ether in light petroleum and tocopherol by 6% (v/v) of ether in light petroleum, but the separation was not always sharp. Any remaining tocopherol was eluted by means of 8% (v/v) of ether in light petroleum. Cholesterol, together with substance SC, 7-dehydrocholesterol and vitamin A were eluted by 20% (v/v) of ether in light petroleum. It was important to persist until the eluate (after removal of solvent) ceased to

give a positive Liebermann-Burchard test. Ether (100%) eluted small quantities of material containing any xanthophylls and α -tocopherylquinone.

The sterol fraction was then acetylated (pyridine and acetic anhydride) and the mixture of acetates chromatographed. Most of the cholesteryl acetate was eluted by 1% (v/v) of ether in light petroleum. This fraction also contained 7-dehydrocholesteryl acetate and vitamin A acetate. As soon as the eluate ceased to give a positive Liebermann-Burchard test the eluting solvent was changed to 4% (v/v) of ether in light petroleum and SC acetate came through the column. Elution of this material was completed by means of 8% of ether in light petroleum; 100% ether eluted SC alcohol.

Determination of tocopherol. Tocopherols are sensitive to alkali (Moss, Cuthbertson, Danielli & Drummond, 1938) but saponification of oils in the presence of pyrogallol (Tosic & Moore, 1943) diminishes the loss. Quaife & Dju (1949) determined tocopherol in tissue by first extracting the lipid and then concentrating the vitamin E by molecular distillation. It was important in the present work to know how much tocopherol was destroyed in the alkaline digestion of kidney tissue, how much survived the chromatography, and which were the principal decomposition products.

The method of Emmerie & Engel (1938) was applied to chromatographic fractions which showed signs of selective absorption in the region 290–300 $\text{m}\mu$.

Portions of 0.5, 0.75, 1.0, 1.5, 2.0 and 2.5 ml. of a standard solution of α -tocopherol (Roche Products Ltd.) in ethanol (0.24 mg./ml.) were measured into separate 25 ml. volumetric flasks. To each was added 1 ml. of FeCl_3 solution (0.2%, w/v, in absolute ethanol) and 1 ml. of α -dipyridyl solution (0.5%, w/v, in absolute ethanol, freshly prepared). The volume was made up to 25 ml. with ethanol and after 30 min. the intensity of absorption of each solution was measured at 520 $\text{m}\mu$ with a compensating cell containing a similar solution but with omission of the tocopherol. The calibration curve was a straight line passing through the origin, and if x = mg. of tocopherol and E = $\log I_0/I$ for a 1 cm. cell at 520 $\text{m}\mu$, $x = 0.8E$.

RESULTS

Trial experiments

Experiment 1. A large 'normal' kidney was cut longitudinally and laterally to give four nearly equal parts. One portion was saponified in the usual way and the unsaponifiable matter was chromatographed. A second portion was saponified in the presence of 0.4 g. of pyrogallol and the unsaponifiable matter chromatographed. Tocopherol was added in known amounts to the third and fourth portions, which were then treated in the same way as the first two.

Table 1 (a) shows that the recovery of added tocopherol is far from satisfactory. It is interesting to note that the yield of ubiquinone is about doubled by the use of pyrogallol but that of substance SC is unaffected.

Experiment 2. A 'normal' kidney was divided longitudinally into two equal parts and to each was

added the appropriate volume of 60% KOH, pyrogallol solution and 4.5 mg. of tocopherol. Both were digested on the water bath until the tissue had all dissolved (8 min.). One portion was extracted at once and the other was refluxed with absolute ethanol before extraction. In both cases the tocopherol found was about 57% of that added. There was no difference in the ubiquinone content observed. From this experiment it was clear that the loss of tocopherol occurred at an early stage and was not made worse by refluxing with ethanol.

Experiment 3. A large 'normal' kidney was divided longitudinally and laterally to give four nearly equal parts. In this test the point at issue was whether contact with concentrated aqueous alkali is the destructive agency. Table 1 (b) shows beyond doubt that the 60% KOH and the ethanol (containing pyrogallol) must be mixed first and that undiluted 60% KOH is unsuitable. The

recovery of added tocopherol can now be raised to 88%. This procedure seems also to permit optimum extraction of ubiquinone and substance SC.

Experiment 4. It was found that tocopherol was absent from the human kidney cortex, but the technique used probably involved considerable loss of tocopherol. Accordingly, the findings of expt. 3 were applied to a further test of this point. A 'normal' kidney was divided longitudinally into two parts, one of which was dissected to give medulla and cortex. The other half was 'saponified' whole. The alkali, premixed with ethanol containing pyrogallol, was added to the tissue in each case. The unsaponifiable fractions were chromatographed; elution with 4% (v/v) of ether in light petroleum removed ubiquinone and tocopherol was eluted with 6% and 8% (v/v) of ether in light petroleum. Tocopherol was found in the fractions from medulla and from the half-kidney but not in the cortical extracts ($\lambda_{\max}$. 292 and 300 m μ). The

Table 1

(a) *Effect of pyrogallol as antioxidant on the analysis of kidney tissue with and without added tocopherol*

Four portions of one 'normal' kidney were used. Arbitrary units: $E_{1\text{ cm}}^{1\%}$. 272 m μ (ubiquinone) or 275 m μ (substance SC) multiplied by weight of chromatographic fraction in mg. If $E_{1\text{ cm}}^{1\%}$ (272 m μ) is 170 for ubiquinone, 1 unit = 6 μ g. of ubiquinone; if $E_{1\text{ cm}}^{1\%}$ (275 m μ) is 90 for 'pure' substance SC, 1 unit = 11.1 μ g. of substance SC.

Wt. of tissue (g.)	Pyrogallol added (g.)	Tocopherol added (mg.)	Tocopherol found (mg.)	Ubiquinone found (units/g. of tissue)	Substance SC found (units/g. of tissue)
42	Nil	Nil	0.22	2.16	1.0
41	0.4	Nil	0.30	4.01	0.96
40	Nil	3.8	1.72	2.18	0.97
40	0.4	4.0	2.24	4.32	0.95

(b) *Effect of saponification procedure, with four portions of the same kidney*

(i) Tissue digested with 60% KOH containing pyrogallol, followed by refluxing with ethanol for 30 min. (ii) Tissue digested under reflux for 30 min. with premixed 60% KOH and ethanol and pyrogallol. (iii) α -Tocopherol added; digestion was as described in (i). (iv) α -Tocopherol added, digestion was as described in (ii).

Portion	Wt. of tissue (g.)	Pyrogallol added (g.)	Tocopherol added (mg.)	Tocopherol found (mg.)	Recovery of added tocopherol (%)
(i)	38	0.4	Nil	0.30	—
(ii)	37	0.4	Nil	0.87	—
(iii)	37	0.4	4.8	2.74	50.8
(iv)	39	0.4	4.8	5.09	88

Table 2. *Concentrations of tocopherol in human kidney cortex and medulla and whole kidney*

One kidney was divided longitudinally; one half was divided into cortex and medulla.

	Cortex 37	Medulla 31	Whole tissue 70
Wt. of tissue (g.)			
Tocopherol (mg.)			
in 6% ether-light petroleum fraction	Absent	0.20	0.18
in 8% ether-light petroleum fraction	Absent	0.60	0.65
		0.80	0.83
Concn. (mg./100 g.)	—	2.54	1.18

amounts of tocopherol were determined by the Emmerie-Engel method (Table 2). The results confirm the absence of tocopherol from the cortex of the 'normal' human kidney.

'Normal' subjects

The first stage in the present study was to examine human kidney tissue from ten subjects. This was followed by the examination of kidney tissue from four other subjects so as to reveal any differences between cortex and medulla. Table 3 gives the age, sex and cause of death for the subjects, with the analytical findings on the kidneys. Table 4 gives the results for whole kidney cortex and medulla from four subjects. [The third kidney studied gave intractable emulsions during the extraction process; the results obtained are included in the table but were not used in obtaining average values.]

It will be seen that the percentage of unsaponifiable matter (average 0.33) and the vitamin A content (average 3.5 i.u./g.) vary over quite narrow ranges. Ubiquinone is present in every case (average 3.65 units/g. or perhaps 1.5-3.0 mg./kidney) and so is substance SC (average 1.2 units/g.). Again the ranges are narrow, but the result from a boy aged 7 years killed in an accident is exceptional; here substance SC was just detectable. Tocopherol was also found in every case, but from the results it seems certain that the average figure of 0.48 mg./100 g. of kidney is too low and that if the improved method of digestion of tissue had been used throughout, 1 mg./100 g. of kidney tissue would be a better estimate. Mason & Dju (1953) obtained results agreeing better with the revised estimate of about 1 mg./100 g. The sterol content (average 0.23 g./100 g. of tissue) varies, but not very widely.

Abnormal kidneys

Sixteen specimens of kidney tissue from subjects with kidney disease have been studied. Table 5 records the results, the specimens being listed in the order that they were received.

In every case the kidney tissue was treated according to the revised procedure whereby destruction of tocopherol was minimized. The kidney was cut into small pieces, and an equal weight of absolute ethanol was added. Pyrogallol solution (0.5%, w/v, in ethanol) was then added (1 ml./5 g. of tissue). Then 60% (w/v) KOH was added (0.5 ml./g. of tissue). The whole was refluxed on the water bath for 45 min., after which it was cooled, water added and extraction with ether carried out in the ordinary way.

The condition shown in the table for each specimen was assigned to it by the pathologist concerned, in the light of the clinical history and (usually) of the histological findings. Three types were studied, namely chronic, acute and sub-acute chronic (large white kidney) nephritis. Chronic nephritis

Table 3. 'Normal' human kidneys

No.	Sex and age (years) of subject	Cause of death	Wt. of kidney (g.)	Percentage of unsaponifiable matter	Vitamin A (i.u./g.)	Ubiquinone (units/g. of kidney)*	Substance SC (units/g. of kidney)*	Tocopherol (mg./100 g. of kidney)	Sterol (g./100 g. of kidney)
1	M.	57	140	0.272	2.8	3.24	1.71	0.72	0.165
2	F.	44	108	0.301	4.7	2.00	1.20	0.80	0.127
3	M.	59	133	0.125†	1.3	0.80	0.95	0.11	0.093
4	M.	59	112	0.32	1.7	4.95	1.43	0.34	0.173
5	F.	75	138 (two)	0.309	2.2	3.67	0.62	0.36	0.251
6	M.	51	89	0.29	2.1	4.07	1.96	0.41	0.195
7	F.	56	102	0.30	6.8	2.07	1.24	0.40	0.220
8	F.	72	75	0.39	3.9	3.21	0.91	0.61	0.261
9	F.	20	150	0.24	4.0	1.70	0.27	0.56	0.181
10	F.	63	116	0.39	3.9	4.70	1.80	0.73	0.284
11	M.	78	77	0.395	3.6	5.10	1.18	0.60	0.271
			85	0.375	3.0	4.00	0.68	0.50	0.245
12	M.	63	127	0.34	6.2	4.50	2.37	0.37	0.257
13	M.	52	55.5	0.34	1.7	3.90	0.85	0.37	0.241
14	M.	7	40	0.396	2.7	4.00	Trace only	0.29	0.272
				0.33	3.5	3.65	1.2	0.48	0.23

† Low yield was due to intractable emulsions.

* See Table 1 for explanation of units.

Table 4. *Comparison of the unsaponifiable constituents from whole human kidney and from kidney cortex and medulla*

One kidney from each of subjects 11, 12, 13 and 14 was analysed whole, the other after separation into cortex and medulla; from the latter results, the values for the whole second kidney were calculated (column 3). The results for each set of four kidneys have been averaged.

	Whole kidney	Calc. for other kidney	Cortex	Medulla
Wt. of tissue (g.)	300.5	301.5	152.5	149
Percentage of unsaponifiable matter	0.368	0.369	0.395	0.344
Vitamin A concentration (i.u./g.)	3.5	2.5	3.4	1.8
Percentage of unsaponifiable matter recovered after chromatography	84	79	80	77
Ubiquinone (units/g.)	3.8	4.7	5.6	3.8
Substance SC (units/g.)*	1.5	1.2	1.7	0.8
Tocopherol (mg./100 g. of tissue)	0.42	0.37	Absent	0.74
Sterol (g./100 g. of tissue)	0.26	0.26	0.27	0.26

* Excluding subject no. 14 (Table 3), where substance SC was present in negligible amount.

is the most common of the three conditions, but we were fortunate in obtaining good specimens of kidneys characteristic of the other two states. Three cases may be described as examples.

Subject 15 was a man aged 46, who died of sub-acute chronic nephritis. The kidney was big and the cortex was very light in colour and enlarged. The medulla was dissected from the cortex and the two tissues were examined separately.

When cortical unsaponifiable matter (209 mg.) was chromatographed, light petroleum eluted 5.4 mg. of material showing an inflexion near 260 $m\mu$ and 2% (v/v) of ether in light petroleum eluted 1.8 mg. of material containing carotene. When 4% (v/v) of ether in light petroleum was used, a small amount (0.5 mg.) of material with λ_{max} 270 $m\mu$ was eluted and the absorbing entity was found to be cholesta-3:5-dien-7-one (Kantiengar & Morton, 1955). When the eluting solvent was 6% (v/v) of ether in light petroleum ubiquinone was removed. The vitamin A and cholesterol (177 mg.) were eluted by means of 20% (v/v) of ether in light petroleum. Ether eluted a further 13 mg. of material. The sterol fraction was esterified with pyridine and acetic anhydride and the acetate was rechromatographed. The fractions contained 7-dehydrocholesterol and vitamin A in small traces but substance SC was not found.

Medullary unsaponifiable matter (202 mg.) was similarly studied. Ubiquinone was found but no cholesta-3:5-dien-7-one. The fraction (3.6 mg.) eluted by means of 6% (v/v) of ether in light petroleum showed λ_{max} 290 $m\mu$ with a secondary peak at 298 $m\mu$ ($E_{1\%}^{1\text{cm}}$ 7.08, 6.87). The absorbing substance was tocopherol. A further, smaller amount of tocopherol was eluted by means of 8% (v/v) of ether in light petroleum. With 20% (v/v) of ether in light petroleum cholesterol (152 mg.) was eluted together with traces of 7-dehydrocholesterol and vitamin A. Ether eluted 1 mg. of additional adsorbate. Esterification of the sterol

fraction, followed by chromatography, showed substance SC to be absent. The amounts of ubiquinone found were 1.64 and 1.0 unit/g. of medulla and cortex respectively. Substance SC was absent from both types of tissue and tocopherol found to the extent of 0.92 mg./100 g. of medulla was absent from the cortex. A trace of dienone was found in the cortex but not in the medulla.

Subject 16 was a woman aged 79 who died of chronic nephritis. Although the demarcation of cortex and medulla was ill-defined, the two tissues were separated as well as was possible and they were studied separately.

Cortical unsaponifiable matter (92 mg.) was chromatographed in the usual way. The sole absorbing entity eluted by 4 and 6% (v/v) of ether in light petroleum was ubiquinone. The sterol eluted by 20% (v/v) of ether in light petroleum was acetylated and rechromatographed. Traces of 7-dehydrocholesterol and vitamin A were found but substance SC was absent. The ubiquinone contents were 3.6 and 4.14 units/g. for the medulla and cortex respectively.

Subject 17 was a woman aged 55; there was some arteriosclerosis of the kidneys but no nephritis. The kidney was cut longitudinally and one half was divided into medulla and cortex. Chromatography of the half-kidney unsaponifiable matter (440 mg.) gave with 4% (v/v) of ether in light petroleum a good ubiquinone fraction [2.2 mg., $E_{1\%}^{1\text{cm}}$ (272 $m\mu$) 85.5] and with 6% (v/v) of ether in light petroleum the absorbing material contained ubiquinone and tocopherol. The main part of the tocopherol (λ_{max} 290 and 300 $m\mu$) was, however, eluted with 8% (v/v) of ether in light petroleum. The sterol fraction, eluted by means of 20% (v/v) of ether in light petroleum, was examined and found to contain a little substance SC and vitamin A; it was then acetylated and rechromatographed. The major part of the material was eluted with 1% (v/v) of ether in light petroleum and this contained

Table 5. *Abnormal human kidneys*

No.	Sex and age (years) of subject	Diagnosis	Wt. of kidney (g.)	Percentage of unsaaponifiable matter	Vitamin A (i.u./g. of tissue)	Ubiquinone (units/g. of kidney)†	Substance SC (units/g. of kidney)†	Tocopherol (mg./100 g. of kidney)	Sterol (g./100 g. of tissue)	Tocopheryl- quinone (mg./100 g. of kidney)
15	M.	46 Subacute chronic nephritis; large kidney, pale enlarged cortex	195	0.56	0.4	1.3	Absent	0.46	0.383	Absent
16	F.	79 Chronic nephritis	43	0.55	6.7	3.87	Absent	Trace	0.315	Absent
17	F.	55 Some arteriosclerosis of kidney	72	0.43	2.8	8.2	1.35	0.75	0.292	0.75
19	M.	68 Uraemia, chronic nephritis	51	0.35	2.1	2.27	0.45	1.27	0.205	Absent
20	F.	— Acute nephritis*	143	0.35	1.0	2.52	Absent	Absent	0.244	0.14
21	F.	— Acute nephritis*	144	0.55	1.2	1.55	Absent	Absent	0.301	0.20
22	M.	66 Chronic nephritis and pneumonia	73	0.29	0.6	2.16	Absent	Trace	0.183	0.41
23	F.	55 Chronic nephritis; kidneys very small	41.5	0.42	2.9	2.70	Absent	Absent	0.249	0.44
24	M.	3 Sub-acute chronic nephritis; large pale kidneys, typical	80 (two)	1.06	1.2	4.97	Absent	0.46	0.548	Absent
25	M.	50 Chronic nephritis*	87	0.43	2.6	3.69	Absent	1.28	0.350	Absent
26	M.	39 Chronic glomerular nephritis*	125 (two)	0.40	1.5	3.82	Absent	1.82	0.269	Absent
27	M.	67 Sub-acute chronic nephritis	165	0.61	2.0	5.4	Absent	0.86	0.508	Absent
28	M.	73 Chronic hypertensive nephritis	109 (two)	0.32	0.9	4.77	Absent	1.92	0.215	Absent
29	M.	80 Mild chronic hypertensive nephritis with granular scarring and some adhesion of the capsules	152 (two)	0.42	2.0	2.82	Absent	0.70	0.242	Absent
30	F.	42 Lipoid nephrosis	160	1.23	2.2	5.53	Absent	Absent	1.042	0.10
31	M.	42 Terminal chronic nephritis	36	0.46	1.6	7.37	Absent	0.72	0.290	0.15

* Confirmed histologically.

† See Table 1 for explanation of units.

cholesteryl acetate plus traces of 7-dehydrosteroid acetate and vitamin A acetate. The eluate with 4% (v/v) of ether in light petroleum showed no selective absorption. The eluate with 8% (v/v) of ether in light petroleum (1.5 mg.) showed the absorption spectrum of SC acetate practically undistorted. Ether eluted a small quantity of tocopherylquinone giving twin peaks at 260 and 270 m μ .

The whole process of obtaining unsaponifiable fractions and chromatographic separation was then carried out both on the cortex and medulla and the results are shown in Table 9.

Determinations of the principal trace constituents were made similarly on other diseased kidneys.

The most striking result (Table 5) is that substance SC is absent from the kidney in all but one of the cases of nephritis. Tocopherol is sometimes absent and sometimes present at concentrations which are not below the normal (say 1 mg./100 g.). When tocopherol is absent, tocopherylquinone can often be detected (five cases).

DISCUSSION

Lowe *et al.* (1957) found that ubiquinone and substance SC were normal constituents of the unsaponifiable matter from kidneys of 11 species, the amounts present being of the order 20 μ g./g. for the former substance and 5 μ g./g. for the latter. For 13 'normal' human kidneys and 15 nephritic kidneys the average ubiquinone content of 3.65 and 3.64 units/g. respectively are almost identical and correspond with 21.9 μ g./g. This agrees very well with the average for other species and it is clear that not only is the range of variation small but ubiquinone also does not vary in kidney disease.

Kidney tissue in most species contains from 1 to 10 i.u. of vitamin A/g. Moore & Sharman (1950) noted that in rats receiving 40 i.u. of vitamin A/day, males but not females consistently stored more vitamin A in the kidneys than in the liver. This is perhaps a special situation, and in fact kidney stores are in most species quite low when liver stores are high. The differences in vitamin A content shown in Tables 4, 5 and 8 are quite small and afford little indication of a direct relationship between kidney disease and kidney vitamin A. The report by Lawrie, Moore & Rajagopal (1941) that nephritic kidneys do not contain vitamin A is not confirmed (Tables 6 and 7).

Lowe *et al.* (1957) found vitamin E in cat, dog and sheep kidney, the concentration in cat kidney being very high compared with the other two species. The total lipid content of cat kidney is high compared with other species (Mottram, 1916; Turner, 1931). Gavins & Morrison (1949) found

considerable amounts of lipids in the tubule cells of the nephrons of cats, both by chemical and histological methods. In the cat both vitamins A and E occurred predominantly in the cortex; indeed no vitamin E could be found in the medulla. Vitamin E is present in human kidney to the extent of about 1 mg./100 g., as compared with perhaps 2.5 mg./100 g. for cat kidney. In the human kidney the tocopherol is located in the medulla and none can be found in the cortex (Tables 4 and 9). Tocopherol was detected, in fairly normal amounts, in the kidneys of six out of nine subjects with chronic nephritis. In two cases of acute nephritis no tocopherol could be found in the kidney unsaponifiable matter but quite appreciable amounts of tocopherylquinone were found instead. The pattern of incidence of this quinone in kidney extracts is not easy to explain but it is not, in our view, consistent with the substance being a laboratory artifact of tocopherol.

Table 6. *Comparison of kidney cortex and medulla in a case of sub-acute chronic nephritis*

	Cortex	Medulla
Weight (g.)	130	65
Percentage of unsaponifiable material	0.579	0.519
Vitamin A (i.u./g. of tissue)	0.4	0.4

Table 7. *Comparison of kidney cortex and medulla in a case of chronic nephritis*

	Cortex	Medulla
Weight (g.)	22	21
Percentage of unsaponifiable material	0.465	0.629
Vitamin A (i.u./g.)	7.3	6.0

Table 8. *Comparison of kidney cortex and medulla in a case of arteriosclerosis of kidney*

One kidney was divided into two; one half was divided into cortex and medulla.

	Whole tissue	Cortex	Medulla
Weight (g.)	36	—	—
Percentage of unsaponifiable material	0.434	0.51	0.40
Vitamin A (i.u./g.)	2.8	3.8	1.6

Table 9. *Minor constituents of unsaponifiable matter from cortex and medulla of an arteriosclerotic kidney (subject no. 17)*

	Cortex	Medulla
Ubiquinone (units/g.)*	8.9	7.4
Substance SC (units/g.)*	1.5	1.2
Sterol (g./100 g.)	0.306	0.276
Tocopherol (mg./g.)	Absent	1.5
Tocopherylquinone	Absent	Present

* See Table 1 for explanation of units.

Table 10. *Comparison of the unsaponifiable constituents of 'normal' kidneys and abnormal kidneys of human*

	Percentage of unsaponifi- able material	Vitamin A (i.u./g.)	Ubiquinone (units/g.)*	Substance SC (units/g.)*	Tocopherol (mg./100 g.)	Sterol (g./100 g.)
Normal kidneys (thirteen subjects)	0.33	3.5	3.65	1.2	0.48†	0.23
Chronic nephritis (nine subjects)	0.40	2.3	3.72	Absent in eight of nine subjects	Present in six, absent in three out of nine subjects	0.25
Acute nephritis (two subjects)	0.45	1.1	2.0	Absent	Absent	0.27
Sub-acute chronic neph- ritis (large white kidney; four subjects)	0.86	1.4	4.3	Absent	0.44 (absent in one case)	0.62

* See Table 1 for explanation of units.

† 1.0 is a more likely figure (see text).

In the normal kidney Mason & Dju (1953) found that the vitamin E content was greater for females than males. This is confirmed for the present 'normal' series. In Table 5 it is seen that of the five nephritic kidneys obtained from females four lacked tocopherol altogether and the fifth contained only a trace. On the other hand, all the kidneys from males with nephritis contained (with one exception, subject no. 22) normal amounts of tocopherols. So far as the results go, the disappearance of tocopherol from the kidney is associated with nephritis (any type) in the female; the arteriosclerotic kidney from a woman showed a normal tocopherol content. The evidence points to the possibility that in the female tocopherol disappears in nephritis via the tocopherylquinone.

O'Connor (1955) reported that some cases of acute nephritis responded to tocopherol so that kidney-function tests gave normal results. Massive doses were given, but if excessive breakdown of tocopherol is characteristic of nephritis a large intake might well be necessary in compensatory therapy. Butturini (1955) also claims that tocopherol is beneficial in lipoid nephrosis.

Substance SC, according to Lowe *et al.* (1957), is a regular constituent of animal kidney. Human kidney with about 13 $\mu\text{g./g.}$ is the richest source of SC yet known, with the exception of the liver of the vitamin A-deficient rat. From Table 5 it is seen that 14 out of 15 subjects with kidney disease exhibited no SC. In the 'normal' human kidney SC is not sharply localized in the medulla or the cortex.

If substance SC is equally available to all segments of the nephron it may possibly be concerned in maintaining the functioning nephron. Substance SC appears at the age of 1-2 years, when kidney function approaches that of the adult, and disappears in nephritis and nephrosis in which the nephron is affected.

The four cases of sub-acute chronic nephritis, in

which the kidneys were large and white, showed (Tables 5 and 10) markedly elevated percentages of unsaponifiable matter, most of the increase being accounted for by a rise in cholesterol content.

Among the clinical manifestations of nephrosis are oedema, albuminuria, low metabolic rate, lipaemia and hypercholesterolaemia (Leiter, 1931*a*). There is often a rise in blood cholesterol, especially esterified cholesterol (Lichtenstein & Epstein, 1931). Histological study of nephritic kidneys shows lipid deposits made up mainly of cholesterol esters. Leiter (1931*b*) analysed kidneys from seven cases of glomerulonephritis for free and combined cholesterol. The highest figure for free cholesterol (0.297%) was not very high but the esterified cholesterol went up to 0.294%, ten times the normal figure.

It has been shown (Clainesborough, 1929; Heymann & Startzman, 1946) that nephrotic hyperlipaemia is due neither to increased intestinal absorption of cholesterol nor to excessive fat intake. It is more likely to arise from increased mobilization (Heymann, Matthews, Lemm, Olynky, Salehar & Gilkey, 1954; Peters & Van Slyke, 1946). There is often a rise in blood cholesterol which, according to Lichtenstein & Epstein (1931), is mostly in the ester portion, but according to other reports affects the free and esterified cholesterol equally (cf. Thomas, Rosenblum, Lander & Fisher, 1951). London, Sabella & Yamasaki (1951) found that the deuterium content of plasma cholesterol in subjects given D_2O increased more slowly in the nephrotic than in the non-nephrotic subject. Byers, Friedman & Rosenman (1954) and Sadhu & Mookerjee (1955*a*) did not find any marked rise in concentration of liver cholesterol in nephrotic rats, but the rate of cholesterol synthesis in the kidney increased considerably.

Lyman & Barron (1940), using rats with experimental nephritis, found notable depression of oxidative processes in kidney slices with succinate,

lactate, pyruvate and alanine as substrates. Sadhu & Mookerjee (1955*a*) suggest that in nephritis there is a partial blocking of the tricarboxylic acid cycle in the kidney tissue. Accumulation of acetate and acetoacetate might occur in the kidney and this might necessitate a diversion to cholesterol synthesis. The idea gains support from the observation that hypothyroid rats oxidize acetate at a reduced rate and synthesize more cholesterol (Sadhu & Mookerjee, 1955*b*).

The above considerations might well have a bearing on the problems of subacute chronic nephritis, but under the conditions of the present work the sterol contents of 'normal' kidneys and those characteristic of chronic nephritis and acute nephritis are very similar. It would have been attractive to correlate the disappearance of substance SC with an enhanced cholesterol synthesis, but the evidence does not justify it.

SUMMARY

1. The unsaponifiable matter from a variety of specimens of 'normal' human kidney has been studied. The percentage of unsaponifiable matter is about 0.33, of which about two-thirds is cholesterol. The quantitatively minor constituents include vitamin A (about 0.1 mg./100 g.), ubiquinone, substance SC and tocopherol, each about 1 mg./100 g. In all cases when the medulla and cortex were separated by dissection the tocopherol was found to occur only in the medulla. Kidneys from females were on the average richer in tocopherol than those from males.

2. Sixteen specimens of diseased kidney tissue have been studied. The ubiquinone content was similar to that found in 'normal' kidneys. The vitamin A content was not much changed. Tocopherol was not found in nephritic kidneys from females but nephritic kidneys from males contained normal amounts. Tocopherylquinone was often found in nephritic kidneys lacking tocopherol. It is possible that tocopherylquinone is an intermediate in the destruction of vitamin E.

3. Normal human kidney is (next to liver from vitamin A-deficient rats) the best source yet found for substance SC. This substance cannot be detected in diseased human kidneys.

4. In four cases of subacute chronic nephritis, in which the kidneys were large and pale, the percentages of unsaponifiable matter and in particular of cholesterol were much larger than in 'normal' kidneys. The change was much less marked in chronic nephritis and acute nephritis.

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