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Unsaponifiable Constituents of Kidney in Various Species

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Two substances, designated SA and SC respectively pending determination of their structures, have been found in unsaponifiable fractions from various organs, e.g. liver and intestine, from both vitamin A-deficient and normal rats (Heaton, Lowe & Morton, 1955, 1957). The purpose of the present investigation was to study their occurrence in normal kidneys from various species, one of the objectives being to find a convenient source of either SA or SC. In the course of the work a constituent was encountered in some species, particularly cat, which was provisionally denoted SE. It was later identified as vitamin E, probably mainly α -tocopherol. In the domestic cat, the kidney was found to differ from the other species studied in having unusually large amounts of both vitamin A and vitamin E.

The substance SA occurs in the kidneys of all eleven species studied, the concentration being of the order of 20 μ g./g.; it also occurs in human kidney (Mervyn, 1956). The substance SC is almost equally widely distributed, the concentration being of the order of 5 μ g./g. These estimates are based on the properties (in preparation) of the purest preparations of SA and SC so far obtained.

MATERIALS

Kidneys from pig, sheep and ox were obtained as fresh as possible from the Stanley Abattoir, Liverpool. Kidneys from three piglets suspected of suffering from vitamin A deficiency were supplied by Mr Brooksbank, Veterinary Investigation Officer. Dog kidney was obtained from the Veterinary School, University of Liverpool and from the Dogs' Home, Liverpool. Hen kidneys from normal laying pullets were supplied by J. Bibby and Sons Ltd., Liverpool. Cat kidneys were obtained (a) from the Department of Physiology, University of Liverpool, and (b) through the Royal Society for the Prevention of Cruelty to Animals (R.S.P.C.A.), Liverpool Branch. When it became necessary

to obtain larger quantities of material, permission was obtained from W. Smith and Sons, Lymm, to dissect carcasses bought by them from the R.S.P.C.A. The cats were unwanted and had been taken to the Society's Branches by the public and had there been painlessly killed. The dissection was done at W. Smith and Sons' premises. The material was admittedly not all entirely fresh, but no other method of obtaining adequate supplies was possible. Rabbit kidney was obtained from laboratory animals. Two batches of mouse kidney were obtained, one through the co-operation of the Wellcome Laboratories of Tropical Medicine, London, and the second with the help of The Distillers Co. Ltd., Liverpool. Lasco Ltd., Liverpool, provided kidneys from two healthy horses killed after being injured in accidents. Guinea-pig kidneys were obtained from normal laboratory animals. Kidneys from a goat came from the Department of Veterinary Pathology, University of Liverpool; the animal had been suffering from John's disease, but this is not known to make the kidney abnormal.

The authors wish to record their gratitude for much help in obtaining materials and to acknowledge the courtesy extended to them by many persons acting on behalf of institutions, laboratories or industrial firms.

METHODS

Preparation of unsaponifiable matter. The kidney tissue was subjected to digestion with ethanol and KOH as follows: 60% (w/v) aqueous KOH (0.5 ml./g. of tissue) was added to moist kidney and the mixture was heated on the water bath until it became homogeneous; ethanol (1 ml./g. of tissue) was then added and the mixture was boiled under reflux for 30–45 min. After cooling, water (2 vol.) was added and the solution was extracted 5 or 6 times with ether (freshly redistilled over reduced iron). The combined ether extracts were washed free from alkali, dried over Na_2SO_4 , filtered through a sintered-glass funnel and evaporated to dryness under nitrogen. Any kidneys and any unsaponifiable fractions which it was necessary to store were kept at -20° .

Chromatography. The unsaponifiable matter was chromatographed on alumina (Brockmann Grade 3) in the manner described in previous papers (cf. Heaton *et al.* 1957).

RESULTS

Table 1 indicates the number of batches studied for the different species; it shows how the percentage of unsaponifiable matter varies over a narrow range and how a rather weak absorption peak near 272 $m\mu$ was shown for almost every species. The only exception was cat kidney which exhibits a surprisingly high vitamin A content. Further tests were made on cat kidney later (see p. 233). Table 2 illustrates the chromatographic separation of unsaponifiable constituents from pig kidney. Fraction 3, eluted from alumina (Brockmann 3) by 4 % ether-light petroleum, shows $\lambda_{\max}$ 272 $m\mu$ and an inflexion at 320 $m\mu$ with a very weak band near 400 $m\mu$ (solvent *cyclohexane*), and together with fraction 4 consists very largely of SA. Fraction 6, eluted by 20 % ether-light petroleum, is predominantly cholesterol with some selectively absorbing contaminants. This bulky sterol fraction was acetylated by means of acetic anhydride and pyridine and the product was rechromatographed (Table 3). The least-strongly adsorbed material contains cholesteryl acetate and a trace of vitamin A acetate. The small amount of material eluted by

4 % ether-light petroleum is (by selective absorption) SC acetate mixed with transparent material, e.g. traces of cholesteryl acetate. Fraction 3 eluted by 8 % ether-light petroleum is a similar mixture and contains probably some free SC as well as its acetate.

Table 4 summarizes the results obtained with kidneys from various species. In these experiments the vitamin A content of kidney from mouse, guinea pig and horse was very low but the vitamin is not likely to have been wholly absent. If the vitamin A content of a tissue is of the order 1 i.u./g. special care is needed if a quantitative estimate is to be obtained. In general, kidney contains a few i.u. of vitamin A/g., but the results with cats are quite unusual (see Table 5).

The work on sheep kidney will be reported in more detail. Four batches were examined; the percentage of unsaponifiable matter ranged from 0.33 to 0.58, the vitamin A content from 3.0 to 4.3 i.u./g. and the sterol content ranged from 0.26 to 0.43 %. Each batch showed the presence of carotenoids in traces only and 7-dehydro steroid absorption was just detectable. SA and SC were detected in all cases. A substance SE, which

Table 1. *Kidney unsaponifiable matter*

Species	No. of batches	Combined wt. of tissue (g.)	Average percentage of unsaponifiable matter	Absorption spectrum of unsaponifiable matter (<i>cyclohexane</i>)	$E_{1\text{ cm.}}^{1\%}$ (calc. on unsaponifiable matter)
Pig	3	549	0.48	272-3	2.55
Piglets	3	55.5	0.51	~ 235 ~ 270	5.0 2.1
Sheep	4	435	0.44	272 327	1.6 0.9
Dog	2	143	0.32	~ 280 $\lambda_{\max}$ 300 ~ 327	3.0 2.75 3.3
Rabbit	1	33.5	0.54	272 ~ 280 330	1.69 1.55 0.96
Ox	2	211	0.37	272 330 450	2.4 0.97 1.7
Mouse	2	42	0.42	272 ~ 280	5.7 5.2
Horse	2	221	0.32	272 280	2.26 2.1
Guinea pig	1	32	0.38	272 ~ 320	3.24 0.83
Goat	1	86	0.37	272 ~ 320	1.79 0.72
Cat	5	208	0.52	327	12.7
Hen	1	35	0.36	~ 230 272 327 450	4.7 2.86 2.98 1.14

~ = inflexion.

Table 2. *Chromatography of pig-kidney unsaponifiable matter*

Alumina (Brockmann Grade 3), 20 g. Column, 10 cm. $\times$ 1.7 cm. Wt. of unsaponifiable matter used, 0.190 g.; wt. recovered, 0.167 g.

Fraction	*Eluent	Vol. (ml.)	Wt. of solute (mg.)	Absorption spectrum (cyclohexane) (m μ)	$\dagger E_{1\text{ cm.}}^{1\%}$
1	P	200	2.0	$\sim$ 230 $\sim$ 255	34.7 12.4
2	2% E/P	200	1.6	$\sim$ 235 $\sim$ 255	7.5 6.1
3	4% E/P	300	2.0	$\lambda_{\text{max.}}$ 272 $\sim$ 320 $\lambda_{\text{max.}}$ 400 (flat)	91.5 9.3 4.2
4	6% E/P	200	1.4	$\lambda_{\text{max.}}$ 273–275 $\sim$ 320	65.2 6.2
5	8% E/P	200	1.8	$\lambda_{\text{max.}}$ 275 $\sim$ 325	7.4 1.7
6	20% E/P [Liebermann–Burchard test positive]	900	157.3	$\sim$ 235 $\sim$ 267 $\sim$ 275 $\lambda_{\text{max.}}$ 282 $\lambda_{\text{max.}}$ 327	0.91 0.42 0.45 0.43 0.30
7	E	200	0.6	$\sim$ 230 $\sim$ 270 $\sim$ 320	31.0 14.6 4.6

$\sim$ = inflexion.

* In this and other tables E represents diethyl ether and P represents light petroleum.

$\dagger E_{1\text{ cm.}}^{1\%}$ calculated on the weight of the fraction.

Table 3. *Chromatography of the sterol-rich portion of pig-kidney unsaponifiable fraction after acetylation*

Alumina (Brockmann Grade 3), 15 g. Column, 7.8 cm. $\times$ 1.7 cm. Wt. of acetate used, 0.159 g. Wt. recovered, 0.157 g.

Fraction	Eluent	Vol. (ml.)	Wt. of solute (mg.)	Absorption spectrum (cyclohexane) (m μ)	$E_{1\text{ cm.}}^{1\%}$
1	1% E/P	350	154	$\sim$ 255 $\sim$ 275 $\sim$ 285 $\lambda_{\text{max.}}$ 327	0.25 0.20 0.18 0.15
2	4% E/P	250	0.9	$\sim$ 230 $\lambda_{\text{max.}}$ 275 $\sim$ 282 $\sim$ 315	53.7 23.8 21.9 8.8
3	8% E/P	300	0.6	$\sim$ 230 $\lambda_{\text{max.}}$ 275 $\sim$ 282 $\sim$ 325	89.1 38.1 35.4 14.2
4	E	200	1.0	$\sim$ 230 $\sim$ 255 $\sim$ 280	62.5 36.8 22.9

$\sim$ = inflexion.

Table 4. *Comparison of various species in respect of certain constituents of unsaponifiable material from normal kidneys*

Results are expressed as amounts/gram of kidney tissue unless otherwise stated.

Species	Vitamin A (i.u./g.)	*Tocopherol (mg./g.)	Sterol (g./100 g.)	7-Dehydro steroid	SA† (u./g.)	SC† (u./g.)
Pig	4.9	a	0.29	Tr.	2.8	0.8
Piglets with low liver vitamin A	6.6	a	0.25	a	a	0.55
Sheep	3.6	0.0041	0.32	Tr.	1.3	0.34
Dog	11.7	0.008	0.22	a	3.0	0.30
Cat	98.5	0.025	0.28	Tr.	3.1	0.21
Rabbit	3.7	a	0.35	Tr.	3.0	0.64
Ox	1.37	a	0.29	Tr.	2.3	0.20
Mouse	a	a	0.28	a	8.6	0.74
Horse	a	a	0.29	Tr.	2.2	a
Guinea pig	a	a	0.26	Tr.	4.3	a
Goat	1.25	a	0.28	Tr.	1.9	0.54
Hen	12.7	a	0.22	Tr.	3.0	0.79

a = not detected.

Tr. = trace.

* If small amounts of tocopherols were present they may have been destroyed.

† Arbitrary units as defined in text (see Discussion); if $E_{1\text{cm}}^{1\%}$ (272 m μ) for SA is 170, one unit = 6 $\mu\text{g.}$, and if $E_{1\text{cm}}^{1\%}$ (275 m μ) for SC = 90, one unit = 11.1 $\mu\text{g.}$ Table 5. *Unsaponifiable fractions from cat kidney*

Kidneys from nine cats were used. The vitamin A contents were calculated from ultraviolet absorption in the region 310–340 m μ shown by the unsaponifiable matter. The three-point correction procedure (Morton & Stubbs, 1942; British Pharmacopoeia 1953) was applied, and the results are expressed as i.u./g. of kidney, wet wt.

Expt.	Wt. of kidney tissue (g.)	Unsaponifiable matter (%)	Vitamin A content* (i.u./g.)
1	30	0.72	74
2	20	0.43	69
3	14.5	0.59	103
4	31.2	0.52	134
5	112	0.34	112

Table 6. *Chromatography of sheep-kidney unsaponifiable matter*Wt. used, 177 mg. Alumina column, 10 cm. $\times$ 1.7 cm. Alumina, 20 g. (Brockmann 3).

Fraction	Eluent	Vol. (ml.)	Wt. of solute (mg.)	Absorption spectrum (cyclohexane) (m μ)	$E_{1\text{cm}}^{1\%}$	Remarks
1	P	200	1.3	$\sim$ 255	19	—
2	2% E/P	200	1.7	$\sim$ 230 $\sim$ 260 $\sim$ 330 $\sim$ 425 $\lambda_{\text{max.}}$ 450 $\sim$ 475	11.8 6.8 1.7 1.1 1.3 1.0	— — — Carotenoid — —
3	4% E/P	200	0.2 (?)	$\lambda_{\text{max.}}$ 272 $\sim$ 315	164 24.5	SA —
4	6% E/P	200	0.2 (?)	$\lambda_{\text{max.}}$ 273 $\sim$ 300	160 39	SA —
5	8% E/P	200	1.9	$\lambda_{\text{max.}}$ 290 $\sim$ 295	6.6 6.2	Vitamin E —
6	12% E/P	100	0.1 (?)	$\sim$ 280	44	—
7	20% E/P	800	109	$\sim$ 235 $\sim$ 275 $\lambda_{\text{max.}}$ 285 $\sim$ 320 $\lambda_{\text{max.}}$ 327	0.69 0.38 0.40 0.56 0.59	— — SC — —
8	E	200	0.4	$\sim$ 230 $\lambda_{\text{max.}}$ 275 $\sim$ 285	54 27 25	— — —

Table 7. *Chromatography after acetylation of the main sterol fraction from sheep-kidney unsaponifiable matter (fourth batch)*Wt. used, 156 mg. Alumina column, 7.6 cm. $\times$ 1.2 cm. Alumina, 10 g. (Brockmann 3).

Fraction	Eluent	Vol. (ml.)	Wt. of solute (mg.)	Absorption spectrum (cyclohexane) ($m\mu$)	$R_1^{1\%}$ 1 cm.	Remarks
1	1% E/P	250	153.7	$\sim$ 320 $\lambda_{\max}$ 327	0.35 0.37	—
2	4% E/P	300	0.9	$\sim$ 230 $\lambda_{\max}$ 275 $\sim$ 282 $\sim$ 315	43.5 21.1 19.6 8.3	— SC — —
3	8% E/P	200	0.2 (?)	$\lambda_{\max}$ 232 $\lambda_{\max}$ 275 $\sim$ 282 $\sim$ 320	103 52 50 23.5	— SC — —
4	E	200	0.4	$\sim$ 230 $\sim$ 270 $\sim$ 285	78 42 35	— — —

 $\sim$ = inflexion.Table 8. *Chromatography of cat-kidney unsaponifiable matter*Wt. used, 76.4 mg. Alumina column, 7.7 cm. $\times$ 1.3 cm. Alumina, 10 g. (Brockmann 3).

(a)

Fraction	Eluent	Vol. (ml.)	Wt. of solute (mg.)	Absorption spectrum (cyclohexane) ($m\mu$)	$R_1^{1\%}$ 1 cm.
1	P	100	3.1	$\lambda_{\max}$ { 227 260 350 370 390	30.0 8.66 1.38 1.45 1.06
2	2% E/P	100	2.4	$\sim$ 240–290	5.05
3	4% E/P	100	0.2	$\lambda_{\max}$ 272 $\sim$ 320	168 26.5
4	6% E/P	100	1.8	$\lambda_{\max}$ 292 $\sim$ 295	16.95 16.6
5	8% E/P	50	1.9	$\lambda_{\max}$ 292	4.32
6	20% E/P	300	45.7	$\sim$ 230 $\sim$ 320 $\lambda_{\max}$ 327	33.3 90.7 95.2
7	E	100	0.9	$\sim$ 230 $\sim$ 245 $\lambda_{\max}$ 280	31.9 23.8 19.05

(b) *Rechromatography of fraction 6 after acetylation.* Alumina column, 7.8 cm. $\times$ 0.9 cm. (5 g.).

1	1% E/P	175	47.3	$\sim$ 260 $\sim$ 320 $\lambda_{\max}$ 327	1.90 6.53 6.78
2	4% E/P	150	0.8	$\sim$ 230 $\lambda_{\max}$ { 277 285 $\sim$ 310–320	32.8 22.5 SC 22.5 14.1
3	8% E/P	150	0.5	$\lambda_{\max}$ 232 $\sim$ 279 $\lambda_{\max}$ 285	59.0 54.8 55.7
4	E	100	1.0	$\sim$ 230 $\lambda_{\max}$ 260 $\sim$ 270	20.9 17.55 17.50

 $\sim$ = inflexion.

turned out to be a tocopherol (probably α), was also detected. Table 6 illustrates the chromatographic separation.

It will be seen that following SA there is a fraction (5) with $\lambda_{\max}$. 290 $m\mu$ due to tocopherol. Table 7 shows how the presence of SC was verified by rechromatography of the acetylated sterol fraction from the fourth batch of sheep kidney.

Table 8 illustrates the chromatographic separation conducted on unsaponifiable matter from cat kidney. Fraction 3 is a typical SA fraction and the principal absorption peak in fractions 4 and 5 occurs at 292 $m\mu$. Fraction 6 shows that although sterol is the major constituent in respect of weight the absorption spectrum is very clearly that of vitamin A. The acetylated sterol fraction was rechromatographed and the vitamin A acetate absorption reappears in the least polar fraction as expected. SC was perhaps present in fraction (b) 2 which contained an interfering vitamin A derivative and the absorption of fractions (b) 3 and (b) 4 indicates the presence of other substances in small amounts.

As cat kidney was the best source of the substance showing $\lambda_{\max}$. 292 $m\mu$, $\lambda_{\min}$. 255–260 $m\mu$ (in *cyclohexane*), it was decided to use this tissue in order to establish the nature of the compound. Table 9 indicates how one batch was concentrated.

Two lots of cat kidney weighing 460 and 1221 g. respectively were worked up. The unsaponifiable fractions were prepared and chromatographed to concentrate the SE fraction ($\lambda_{\max}$. approx. 290 $m\mu$). The best fractions were combined and partitioned between light petroleum and 85 % ethanol (15 vol. of water, 85 vol. of ethanol). The best fraction obtained showed $\lambda_{\max}$. 297 $m\mu$, $E_{1\text{cm}}^{1\%}$. 51.5, persistence ($E_{\max}/E_{\min}$.) 4.2. The concentrate from the second batch was acetylated and rechromatographed, giving one fraction exhibiting three maxima at 278,

284 and 287 $m\mu$ with $E_{1\text{cm}}^{1\%}$. 10.55, 11.86, 11.64 respectively. The spectrum corresponded exactly with that of α -tocopheryl acetate diluted with optically transparent material.

α -Tocopherol was obtained by saponifying α -tocopheryl acetate (synthetic) in the presence of pyrogallol. It showed in *cyclohexane* $\lambda_{\max}$. 297 $m\mu$, $\lambda_{\max}$. 291–292 $m\mu$ and $\lambda_{\min}$. 257–258 $m\mu$ ($E_{1\text{cm}}^{1\%}$. at 297 $m\mu$, 90.8). The best fraction from cat kidney showed exactly the same spectrum except that the intensity of absorption ($E_{1\text{cm}}^{1\%}$. 51.5 at 297 $m\mu$) was lower, indicating the presence of a transparent congener. α -Tocopheryl acetate in *cyclohexane* shows peaks at 279 and 285 $m\mu$ with a sharp break at 287 $m\mu$ and a minimum at 255 $m\mu$. This curve is exactly reproduced by the acetate of the product from cat kidney. α -Tocopherol was oxidized by means of ethanolic ferric chloride (John, Dietzel & Emte, 1939) to give α -tocopherylquinone ($\lambda_{\max}$. 261 $m\mu$ and 270 $m\mu$, $E_{1\text{cm}}^{1\%}$. 396 and 391 respectively). The best fraction from cat kidney gave the same two bands with $E_{1\text{cm}}^{1\%}$. values 254 and 248. The effect of alkali on tocopherol is first to increase the absorption near 320 $m\mu$ and to reduce that near 290 $m\mu$. There is room for further study of this effect, but the cat kidney product and tocopherol do not behave differently.

There can be no doubt from the foregoing that cat kidney contains vitamin E in relatively large amount.

Distribution of vitamins A and E in cat kidney.

Four batches of cat kidneys were obtained from the Department of Physiology. Individual kidneys were dissected so as to separate medulla and cortex as cleanly as possible. In four experiments the medulla accounted on the average for 22 % of the weight and the cortex for 78 %. In another test the whole kidney was found to contain 4.3 % of lipid soluble in hot ethanol, but only 10.5 % of this lipid occurred in the medulla.

Table 9. *Chromatography on a larger scale of cat-kidney unsaponifiable matter*

Wt. of kidneys, 460 g. Wt. of unsaponifiable matter used, 1.5 g. Alumina column, 10.5 cm. $\times$ 3.5 cm. Alumina, 100 g. (Brockmann 3). If $E_{1\text{cm}}^{1\%}$. (290 $m\mu$) for α -tocopherol is taken as 90 the amount of vitamin E in fractions 4 (a), 4 (b) and 5 is approximately 14 mg. or 3 mg./100 g.

Fraction	Eluent	Vol. (ml.)	Wt. of solute (mg.)	Absorption spectrum (<i>cyclohexane</i>) ($m\mu$)	$E_{1\text{cm}}^{1\%}$.
1	P	500	—	—	—
2	2% E/P	500	} solutes discarded	—	—
3	4% E/P	800		—	—
4 (a)	6% E/P	300	51.6	$\lambda_{\max}$. { 287 297	14.3 13.4
4 (b)	6% E/P	200	22.1	$\lambda_{\max}$. { 290 297	15.0 15.0
5	8% E/P	700	68.7	$\lambda_{\max}$. 287 ~ 295	3.4 3.0

Table 10. *Vitamin A in medulla and cortex of cat kidney*

Expt.	Tissue	Vitamin A	
		Tissue (i.u./g.)	Whole kidney (i.u./g.)
1	Cortex	140	117
	Medulla	36.3	—
2	Cortex	47.7	39.3
	Medulla	8.2	—
3	Cortex	32.6	24.6
	Medulla	Not found	—
4	Cortex	137	117
	Medulla	31.9	—

Table 10 shows that vitamin A is preferentially located in the cortex to an extent greater than would be expected from the distribution of lipid. Expts. 2 and 3 show vitamin A values lower than in the earlier work but still high compared with other species. Vitamin E was not found here in the kidney cortex. The unsaponifiable materials from Expt. 1 were chromatographed and it appeared that fraction 4 (eluted by 6% ether-light petroleum) contained both SA and vitamin E. The solute was dissolved in a little methanol and left at -20° for 1 hr. The solution was filtered and the methanol-insoluble material was found to contain SA ($\lambda_{\max}$. 272 m μ) and the methanol-soluble fraction to contain vitamin E ($\lambda_{\max}$. 290 m μ , inflexion at 297 m μ).

The estimated vitamin E content of the whole kidney was 1.05 mg./100 g.; this was all in the cortex. Similarly for Expt. 4 the whole kidney contained 1.31 mg./100 g. and the cortex 1.62 mg./100 g., with negative results for the medulla. Although the two vitamins tend to occur together there are obvious fluctuations from one individual to another in the amounts of vitamins A and E in cat kidney. The absence of vitamin E from the medulla is, however, a striking result worthy of further investigation.

DISCUSSION

Kidney tissue for most species contains only very small amounts of vitamin A. This circumstance makes it easier to achieve a sharp fractionation of kidney unsaponifiable than of liver unsaponifiable matter.

Both SA and SC are present in the kidneys of most of the species studied. The units used in Table 4 are such that for vitamin A, 3 i.u. = 1 μ g.; for SA, 1 unit = 6 μ g.; and for SC, 1 unit = 11 μ g. These

figures are based upon the provisional assumption that our best preparations of SA and SC are not far from pure. Thus pig kidney contains (per gram) about 1.6 μ g. of vitamin A, 16.8 μ g. of SA and 8.8 μ g. of SC.

In cat kidney the vitamin A content on the average is about 33 and the vitamin E content 25 μ g./g. Although there must obviously be variations from one individual to another, the figures obtained are high, and the evidence that no vitamin E can be found in the medulla even when there is a good deal in the cortex suggests that the peculiarities of cat kidney are worthy of much more study.

The broad picture presented here carries the implication that similar work on diseased kidneys will be fruitful. For this it is perhaps best to work on human tissue.

SUMMARY

1. It has been shown that SA and SC (two spectroscopically recognizable compounds of unknown composition) are normal constituents of the unsaponifiable fraction of kidneys from eleven different species; the amounts detected in all cases were of the order 20 μ g./g. for SA and 5 μ g./g. for SC.

2. In general it was found that kidney tissue contains only a few i.u. of vitamin A/g., but the results with cats were quite unusual. It was shown that cat kidneys contain an average of approximately 100 i.u. of the vitamin/g.

3. Vitamin E was detected in cat, dog and sheep kidney, the concentration in cat kidney being much greater than in the other two species.

4. A study of the distribution of vitamins A and E in cat kidney revealed that they were to be found predominantly in the cortex. Using the techniques described in the text, vitamin E was not detected in the medulla.

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