SUMMARY

1. Marked differences in the duration of symptoms were observed when sublethal single doses of different dimethyl phosphate esters were injected intravenously into rats. The compounds inducing prolonged symptoms (*OO*-dimethyl *S*-ethylthioethyl phosphorothiolate, *OO*-dimethyl *S*-ethylsulphinyethyl phosphorothiolate and *OO*-dimethyl *S*-ethylsulphonyethyl phosphorothiolate) either persisted themselves, or produced an inhibitor which persisted for several hours in the blood. Those compounds inducing symptoms of short duration (*OO*-dimethyl *S*-ethylsulphonioethylmethyl phosphorothiolate and dimethyl *p*-nitrophenylphosphate) showed short persistence in the blood.

2. *In vivo* the rate of return of brain and erythrocyte cholinesterase activity differed markedly between the two groups of inhibitors. The persistent inhibitors produced irreversible inhibition of practically all of the inactivated enzyme, whereas most of the enzyme inhibited by the non-persistent inhibitors recovered its activity rapidly.

3. There was no distinction between the persistent and non-persistent inhibitors when tested *in vitro* for the return of cholinesterase activity by Aldridge's method. Return of activity took place at the same high rate in all instances, with a half-life of about 1.3 hr.

4. When the non-persistent inhibitor, Me-E 600, was made persistent by slow infusion for several hours into the rat's jugular vein, conversion into the

second irreversible stage of inhibition took place. Decrease in the rate of infusion was followed by a remarkable increase in tolerance of inhibitor, with a parallel increase in production of irreversibly inhibited cholinesterase.

5. Because the conversion from the reversible into the irreversible stage occurs rapidly *in vivo*, it is concluded that both the persistence of the inhibitor and the route, or speed, or both, by which the dimethyl phosphate esters are administered will decide the extent to which this conversion will take place in the animal.

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REFERENCES

- Aldridge, W. N. (1953*a*). *Biochem. J.* **54**, 442.
 Aldridge, W. N. (1953*b*). *Biochem. J.* **53**, 117.
 Church, A. & Ridge, J. W. (1955). *J. Physiol.* **127**, 43 P.
 Davies, D. R. & Green, A. L. (1956). *Biochem. J.* **63**, 529.
 Davison, A. N. (1955). *Biochem. J.* **60**, 339.
 Heath, D. F. & Vandekar, M. (1957). *Biochem. J.* **67**, 187.
 Hobbiger, F. (1956). *Brit. J. Pharmacol.* **11**, 295.
 March, R. B., Metcalf, R. L., Fukuto, T. R. & Maxon, M. G. (1955). *J. econ. Ent.* **48**, 355-363.
 Vandekar, M. (1957). *Biochem. J.* **65**, 1P.
 Wilson, I. B. (1955). *Disc. Faraday Soc.* **20**, 119.

Aspects of Vitamin A Deficiency in the Rat

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McCollum & Davis (1913) and Osborne & Mendel (1913*a*) independently proved that fat-soluble material was indispensable in the diet of the growing rat. Since that time great progress has been made in many aspects of the study of vitamin A, but only in respect of scotopic vision is much known about its mode of action. Research into the systemic mode of action is halted for want of a useful clue.

Lowe, Morton & Harrison (1953), using chromatography and ultraviolet-absorption spectroscopy, detected a new constituent in the unsaponifiable fraction from the livers of vitamin A-deficient rats. The substance, designated SC pending elucidation of its nature, is characterized by absorption maxima at 275 and 332 m μ , with sharp inflexions at 233 and 283 m μ (solvent, cyclohexane).

If SC appears as a direct result of vitamin A deficiency its chemical nature should throw light on processes regulated by the vitamin. If, however, SC occurs as an indirect consequence of vitamin A deprivation its immediate relevance will be less obvious.

The present paper is concerned with comparing (qualitatively and quantitatively) the unsaponifiable fractions of certain tissues from vitamin A-deficient rats and from similar rats given an adequate supplement of vitamin A. (For a preliminary report, see Heaton, Lowe & Morton, 1955.) These experiments lead to conclusions which are, in a limited sense, quite clear-cut, but experiments on chicks and on adult hens and cockerels (Lowe, Morton, Cunningham & Vernon, 1957) showed that

the work on the rat may have uncovered a special manifestation of vitamin A deficiency rather than a general one. At the present stage the need is for exploration and perhaps for establishing that a new approach is possible and worth while.

MATERIALS AND METHODS

Normal rat tissues were obtained from adult animals of the stock colony reared on animal diet no. 4 (British Extracting Co. Ltd., Bromborough Port, Cheshire) supplemented by bread and milk.

Vitamin A-deficient rats were produced by feeding the following diet to weanling animals: casein, 18% (either 'low vitamin content' from Genatosan Ltd. or 'fat-free and vitamin-free' from British Drug Houses Ltd.); rice starch, 65%; arachis oil, 5%; Marmite, 8%; salt mixture (Osborne & Mendel, 1913b), 4%. At first each rat was given approximately 8 g. of food/day; after 3 weeks the allowance was increased to approx. 10 g./day, but towards the end of the experiment the rats did not eat all the food provided. Water was supplied without restriction throughout the experiment.

When after 6-7 weeks on the diet the rats exhibited xerophthalmia and severe loss of weight they were anaesthetized and killed by cardiac puncture. A syringe was used and blood collected (Glover, Goodwin & Morton, 1947).

The control rats were similar weanling animals given the same amount of the vitamin A-deficient diet as the deficient rats and, in addition, a supplement of 20 i.u. of vitamin A/day, administered orally in the form of cod-liver oil of a known potency diluted with arachis oil until the dose was contained in 0.1 ml. of the mixture. After a similar period of 6-7 weeks on the control diet, these rats were anaesthetized, killed by cardiac puncture and dissected.

Saponification and extraction of tissues. Saponification was effected by adding to the moist tissue 60% (w/v) potassium hydroxide (0.5 ml./g. of tissue) and the mixture was heated on a water bath until it became homogeneous. Ethanol (2 vol.) was then added and boiling continued under reflux for 30-45 min. For livers, kidneys, small intestines and submaxillary glands, boiling for 30-45 min. sufficed, but for bladders, vaginas, testes and the skin from around the eyes at least 2-3 hr. refluxing was necessary for complete hydrolysis of tissue. Water was then added and the mixture extracted five or six times with diethyl ether (freshly distilled over reduced iron). The combined ether extracts were washed with water until free from alkali, dried over anhydrous sodium sulphate, filtered through a sintered-glass funnel (G3) and evaporated to dryness under nitrogen.

Chromatography. Chromatographic separations were carried out on alumina (Grade O, P. Spence and Co. Ltd., Widnes) weakened with water to Brockmann Grade 3. Experience showed that with some batches of alumina it was necessary to wash the column with 100-150 ml. of light petroleum to remove an organic contaminant (with ultraviolet absorption showing inflexions at 235 and 260 m μ). This precaution was therefore taken with all batches. The material to be chromatographed was dissolved in the minimum volume of light petroleum and poured on the column. The chromatogram was developed at first with

the same solvent and later with mixed solvents consisting of light petroleum to which had been added certain percentages (v/v) of ether. Eluates were usually examined by the Liebermann-Burchard test or the antimony trichloride test, or both, fractions being cut according to the colours they gave by these procedures. The diethyl ether and the light petroleum (A.R., b.p. 40-60°) used in chromatographic fractionations were dried over sodium wire and redistilled before use, the ether being redistilled over reduced iron. Fractions were taken to dryness under nitrogen and examined spectroscopically.

The total sterol was estimated by weighing the fractions which gave positive reactions in the Liebermann-Burchard test and the 7-dehydro steroid was estimated spectrophotometrically, using a three-point correction procedure described by Glover, Glover & Morton (1952). The absorption curves characteristic of the 7-dehydro-steroid-containing fractions obtained from kidneys did not show the individual peaks (272, 282, 293.5 m μ) clearly enough to permit the use of the above technique, and a correction was made by interpolating a curve for irrelevant absorption in the region 260-300 m μ . By subtracting the irrelevant absorption at 282 m μ it was possible to correct the gross reading and arrive at an approximate intensity for the 7-dehydro steroid contribution.

RESULTS

Altogether 212 weanling rats were placed on the vitamin A-deficient diet, but fourteen died between 5.30 p.m. and 9 a.m. during the course of depletion. These animals were discarded, as it was thought that autolysis occurring over an uncertain period might possibly bring in undesirable complications.

In contrast with reports in the literature that infection of the submaxillary glands often accompanies vitamin A deficiency (e.g. Green & Mellanby, 1928) the glands from only three of the 198 xerophthalmic rats were found to contain pockets of pus visible to the naked eye.

The livers from ten of the worst-affected rats (i.e. those exhibiting the greatest loss of weight or the most severe external eye lesions) appeared abnormal. These livers were distinctly yellow and had small surface nodules. Histological examination showed heavy infiltration with diffusely distributed fat, with a tendency for the centrilobular areas to be less affected than the mid-zones and periportal areas. The lipid was not doubly refractile and seemed to be neutral fat; it occurred in the parenchymal cells and not in the Kupffer cells.

The kidneys from several of the rats with fatty livers displayed hard, greyish green superficial nodules, and kidneys from a few xerophthalmic rats with apparently normal livers were likewise affected. Histological examination revealed, as with the livers, infiltration of neutral fat. The abnormal deposition occurred in the mitochondrial zone of the cells and was restricted to the proximal convoluted tubules.

Some of the deficient rats were found to have a grossly swollen stomach or bladder, the distension appearing to be the result of a blockage at the pyloric sphincter or the exit of the urethra. Sixteen stomachs and thirty-one bladders similar to those shown in Fig. 1 were encountered in the 198 xerophthalmic rats examined. No abnormal livers, kidneys, stomachs or bladders were found in the forty-three control rats and the fifty-one stock colony rats examined in the course of this work.

The gross unsaponifiable materials obtained from the various organs were examined spectroscopically, the results being summarized in Table 1.

Each batch of unsaponifiable material was chromatographed on alumina: a typical separation obtained with the unsaponifiable material from the livers of vitamin A-deficient rats is shown in Table 2. Attention will be focused on fractions 3-7, as these contained substances recognizable by their characteristic absorption spectra. Both fractions 3 and 4

Fig. 1. Vitamin A-deficient rats showing (a) greatly distended urinary bladder, (b) greatly distended stomach.

Table 1. Comparison of organs from stock colony, control and vitamin A-deficient rats

Organ or tissue	Group	No. of rats	Mean wt. of organ or tissue (g.)	Mean vitamin A content (i.u./g.)	Mean % unsaponifiable	Principal absorption peak in cyclohexane (m μ)	$E_{1\%}^{1\text{cm.}}$ calc. (on unsaponifiable matter)
Liver	Stock colony	28	9.82	435	0.322	327	77.6
	Control	43	5.37	11.6	0.412	272	9.2
	Vitamin A-deficient	185	2.77	—	0.499	273-274	16.7
Skin from around eyes	Stock colony	33	0.167	—	2.10	271	3.34
	Control	43	0.072	—	3.06	270	2.17
	Vitamin A-deficient	190	0.077	—	2.49	270	2.29
Kidney	Stock colony	51	1.96	3.2	0.568	273	3.42
	Control	43	1.12	24.0	0.615	274	3.89
	Vitamin A-deficient	150	0.82	—	0.609	272	3.69
Submaxillary gland	Stock colony	33	0.539	—	0.536	272	2.56
	Control	43	0.460	—	0.437	272	2.82
	Vitamin A-deficient	190	0.193	—	0.690	272	3.28
Small intestine	Stock colony	16	9.82	0.15	0.264	272	4.73
	Control	43	2.74	—	0.528	272	2.85
	Vitamin A-deficient	109	3.54	—	0.309	272	3.58
Stomach	Stock colony	8	1.97	—	0.532	282	10.3
	Vitamin A-deficient	27	0.962	—	0.484	282	10.6
Bladder	Stock colony	39	0.135	—	0.641	282	7.36
	Control	43	0.0670	—	0.774	282	8.25
	Vitamin A-deficient	195	0.0470	—	0.472	282	14.9
Vagina	Stock colony	21	0.312	—	0.525	282	6.60
	Control	21	0.137	—	1.12	282	5.23
	Vitamin A-deficient	113	0.075	—	2.08	282	6.97
Testis	Stock colony	11	2.46	—	0.421	270	1.81
	Control	22	2.05	—	0.351	270	5.57
	Vitamin A-deficient	83	0.585	—	0.571	270	3.84

— signifies none found.

Table 2. *Properties of chromatographic fractions from vitamin A-deficient rat-liver unsaponifiable matter*

Unsaponifiable material (304.1 mg.) obtained from 61.1 g. of liver from 23 rats was chromatographed on a column of 30 g. of alumina, length 9.5 cm., diameter 2.1 cm. Column washed with 100 ml. of light petroleum before use. E=diethyl ether, P=light petroleum (see text).

Fraction	Eluent	Wt. of fraction (mg.)	Absorption spectra of fractions (in cyclohexane)	$E_{1\text{ cm.}}^{1\%}$
1	200 ml. of P	34.9	Max. 260–263 m μ	13.7
2	200 ml. of 2% E/P	1.6	Inflexion 260 m μ	40.3
3	300 ml. of 4% E/P	16.9	Max. 272–273 m μ Min. 237 m μ Step-out 330 m μ	176 46.5 12.6
4	300 ml. of 6% E/P	11.0	Max. 272–274 m μ Min. 240 m μ Inflexion 330 m μ	80.0 31.7 8.04
5	200 ml. of 8% E/P	3.0	Inflexion 235 m μ Max. 277 m μ Shoulder 285 m μ Max. 327–330 m μ	54.2 27.8 26.6 9.0
6	200 ml. of 10% + 100 ml. of 12% E/P	12.0	Inflexion 233 m μ Max. 275 m μ Shoulder 283 m μ Max. 332 m μ	107 46.5 45.0 18.4
7	600 ml. of 20% E/P	153.3	Inflexion 235 m μ Max. 275 m μ Shoulder 283 m μ Max. 332 m μ	8.22 3.37 3.16 1.32
8	200 ml. of E	1.1	Max. 272–273 m μ Max. 313 m μ	70.0 30.1

Fig. 2. Ultraviolet-absorption curve for SA in cyclohexane [$E_{1\text{ cm.}}^{1\%}$ (272 m μ), 170].

were yellow and liquid. The substance in fraction 3 responsible for the characteristic absorption spectrum with λ_{max} . 272–273 m μ , λ_{min} . 237 m μ and a step-out at 330 m μ (cyclohexane) is the material previously described by Festenstein, Heaton, Lowe & Morton (1955) and designated SA. The spectrum of fraction 4 differs from that of fraction 3 in the bathochromic displacement of the minimum and the appearance of a sharp inflexion instead of a step-out at 330 m μ (Fig. 2). The substance re-

Fig. 3. Ultraviolet-absorption curves, with cyclohexane as solvent, for SC (---); for SC acetate (—) [SC acetate gave $E_{1\text{ cm.}}^{1\%}$ (275 m μ), 94].

sponsible for the sharp inflexion will be called SB. The chromophoric material in fractions 5–7 (Fig. 3), which has been designated SC, is the

substance first noted by Lowe *et al.* (1953). As can be seen from Table 2, it is very similar in polarity to cholesterol, which was the main constituent of fractions 6 and 7 (positive Liebermann-Burchard reactions).

By cutting fractions as shown in Table 2, satisfactory separations were achieved with the unsaponifiable material from most organs of vitamin A-deficient rats. Not all organs examined, however, contained SA, SB and SC. On the other hand, 7-dehydro steroid was detected in certain organs. When SC and 7-dehydro steroid occurred together, a partial separation was obtained by collecting the sterol-containing eluate in several portions; SC was eluted with the early running portion of the cholesterol, and 7-dehydro steroid accompanied the later running part of cholesterol. A complete separation of these two substances proved difficult.

When livers, kidneys and small intestines from stock colony rats were examined, the vitamin A was eluted in company with the first portion of the sterol fraction and masked the SC. In consequence, a method of separating SC had to be developed and this is shown in Table 3. The absorption spectra of SC acetate and that of the free material are compared in Fig. 3.

It can be seen from Table 1 that in both the liver and bladder, vitamin A deficiency enhances the intensity of absorption ($E_{1\text{cm.}}^{1\%}$) of the gross unsaponifiable material in the range 272–285m μ ,

indicating that the syndrome includes accumulation of substances exhibiting selective absorption. The absorption spectra of the testes gross unsaponifiable materials showed maxima in the regions 230, 270 and 280 m μ and intensities varied with conditions of saponification, suggesting possible alkali isomerization of unsaturated non-conjugated lipids.

To investigate the various changes further, the amounts of SA, SC, 7-dehydro steroid and total sterol have been estimated after chromatography, in these and other organs from vitamin A-deficient, control and stock colony rats. The results are compared in Table 4, a study of which reveals that vitamin A deficiency brings about profound changes. The arbitrary units in which SA and SC contents are expressed were obtained by multiplying $E_{1\text{cm.}}^{1\%}$ at 272 or 275 m μ respectively by the weight (mg.) of the chromatographic fraction. It can be seen that, although SC was obtained from the kidneys of both stock colony and vitamin A-deficient rats, it was not detected in the kidneys from the control animals. When the control rats were examined the technique of methanol precipitation and esterification (Table 3) had not been developed; so it is reasonable to assume that the large amounts of vitamin A in the kidneys of these rats (Table 1) would have masked any small amounts of SC present. Subsequent experience has shown the SC content to be very small indeed.

Table 3. Separation of SC from organs of rats containing vitamin A

Table 4. *Effects of vitamin A deficiency on the concentrations of certain substances occurring in the unsaponifiable fractions of rat organs*Units of absorption are expressed as $E_{1\text{cm.}}^{1\%}$ at 272 or 275 $m\mu$ times wt. of fraction (mg.).

Organ	Group	No. of estimations on which data are based	Mean SA (units/g.)	Mean SC (units/g.)	Mean total sterol (mg./g.)	Mean 7-dehydro steroid (mg./g.)
Liver	Stock colony	2	13.0	0.20	2.03	—
	Control	2	19.9	7.19	2.19	—
	Vitamin A-deficient	8	47.2	15.5	2.78	—
Skin from around eyes	Stock colony	1	—	—	6.90	0.0516
	Control	1	—	—	10.3	0.0371
	Vitamin A-deficient	3	—	—	11.1	0.0725
Kidney	Stock colony	4	12.1	0.516*	3.82	0.0033
	Control	2	10.6	Not detected	4.14	0.0009
	Vitamin A-deficient	4	12.5	0.453	4.08	0.0020
Submaxillary gland	Stock colony	1	3.12	—	3.83	—
	Control	1	4.70	—	3.62	—
	Vitamin A-deficient	3	6.45	—	4.68	0.0019
Small intestine	Stock colony	2	3.43	0.478	1.68	0.00374
	Control	2	2.09	0.651	2.54	0.00950
	Vitamin A-deficient	5	3.23	0.769	2.00	0.00066
Stomach	Stock colony	1	0.80	—	2.67	0.131
	Vitamin A-deficient	1	1.61	—	2.70	0.113
Bladder	Stock colony	2	—	—	1.30	0.063
	Control	1	—	—	2.29	0.076
	Vitamin A-deficient	2	—	—	2.44	0.123
Vagina	Stock colony	1	—	—	1.58	0.061
	Control	1	—	—	2.73	0.028
	Vitamin A-deficient	3	—	—	7.35	0.157
Testis	Stock colony	2	—	—	1.84	—
	Control	2	—	—	1.78	—
	Vitamin A-deficient	4	—	—	3.14	—

— signifies substance sought but not found.

* SC estimated on one batch of kidneys (25 rats) only.

DISCUSSION

Although the occurrence of fat-infiltrated livers was unexpected, it is not the first time that it has been encountered in vitamin A-deficient rats (cf. Monaghan, 1932). However, the fatty kidneys have not previously been reported in avitaminosis A, so far as the authors are aware. Similarly a photograph of a large stomach has been published by Mayer & Krehl (1948), but the distended bladders do not appear to have been previously recorded. Neither the stomachs nor the bladders were examined histologically, but it would appear that sloughing of hyperkeratotic epithelium from the lining of the organ might account for the abnormalities.

Table 4 shows that the concentrations of SA, SC, 7-dehydro steroid and total sterol are all affected by vitamin A deficiency, but the effects vary from one organ or tissue to another. In the livers, submaxillary glands and stomachs, a rise in the content of SA occurs during deficiency; on the other hand,

few changes in the amounts present in the kidney and small intestine were noted. The most prominent change in SC concentration occurs in the liver, where vitamin A deficiency has the effect of causing a large (77-fold) rise. Compared with this, the rise in SC concentration in the small intestine is small. The rise in total sterol is most marked in the vagina and testis, and the submaxillary gland, urinary bladder and vagina exhibit the greatest increases in the concentration of 7-dehydro steroid. In marked contrast is the amount of 7-dehydro steroid found in the small intestine of vitamin A-deficient rats which decreases to about one-sixth of that found in the stock colony rats.

Different tissues have varying degrees of sensitivity towards the accumulation of these substances. With the liver, there is a rise in both SA and SC during vitamin A deficiency, but the concentration of SC is more sensitive to the depletion process than that of SA, which shows a marked increase only near the time when the vitamin A reserves are quite exhausted. A similar effect occurs in the small

intestine, where SC increases slightly during vitamin A deficiency, although the amounts of SA appear to be unaffected.

SA, SC and 7-dehydro steroid could be either normal metabolic intermediates or abnormal products formed as a result of the vitamin deficiency. With the exception of the submaxillary glands (in which 7-dehydro steroid was found only in the deficient animals), these substances have always been detected, though generally in lower concentrations, in the corresponding organs from rats containing vitamin A, thereby indicating that the substances are more likely to be normal metabolic intermediates which accumulate in the deficiency state rather than wholly aberrant products of a deranged metabolism.

Possibly SA and SC are intermediates in different metabolic pathways; the extent to which they accumulate will then depend on the equilibria of the enzymic reactions in the two processes. Alternatively, SA and SC may both be intermediates in the same metabolic pathway. If so, it seems likely that SC is most closely related to the substance whose metabolism is blocked during vitamin A deficiency since, as already pointed out, it is more sensitive in its response to deficiency than SA.

The very selective distribution of 7-dehydro steroid is noteworthy. Originally this substance was regarded principally as a constituent of skin, but Glover *et al.* (1952) showed that it could be formed by desaturation of ingested cholesterol in the small intestine of the guinea pig. The concentrations of this substance in the bladder and vagina of stock colony rats are shown in Table 4 to be greater than in the small intestine and the skin around the eyes of similar rats.

Ward & Moore (1953) have examined the sex organs of the rat and have detected high concentrations of 7-dehydro steroid in the caput epididymis, corpus epididymis and preputial glands of male rats and in the bulbi vestibuli of female rats. They could find no such material in the testes (thus supporting the present results), cauda epididymis, prostates, seminal vesicles or coagulating glands. The occurrence of 7-dehydro steroid at particular sites in the urinogenital tract suggests that it is formed locally and is connected with sexual processes. Morton & Rosen (1949) found the ovaries of pre-hibernation frogs to be yet another specialized locus of 7-dehydro steroid formation. Feyel Cabanes (1949) claims that in rabbits castrated after fertilization the administration of 7-dehydrocholesterol reduces the dose of progesterone required to maintain gestation. The concentration of 7-dehydro steroid in the stomach exceeds that found in any other organ during the present investigation and suggests that the stomach is an important site of sterol metabolism.

The increased concentrations of 7-dehydro steroid observed in the eye tissue, bladder and vagina during vitamin A deficiency are not easy to reconcile with the more spectacular decrease in the concentration of this substance in the small intestine, but appear to indicate that the sterol metabolism of the intestine is quite different from that of the other organs examined. The accumulation of 7-dehydro steroid in the submaxillary gland and vagina is accompanied by the accumulation of spectroscopically transparent sterol, but, in the bladder, vitamin A deficiency appears to exert a much more specific effect on the formation or accumulation of 7-dehydro steroid.

These results indicate that vitamin A is essential for the continuance of certain normal metabolic reactions, but they give no indication of the mode of action. The most obvious suggestion is that it exerts a coenzymic action. From our present limited knowledge of the reactions of the vitamin *in vivo* it appears that oxidation to retinene is the only one which it undergoes readily, suggesting that if vitamin A has a coenzymic function it may be involved in oxidative or reductive reactions such as those of the system vitamin A $\rightleftharpoons$ retinene. The substances whose accumulation has been observed during vitamin A deficiency are unsaturated and hence would appear to be susceptible to either reduction or further oxidation.

SUMMARY

1. The unsaponifiable matter from various organs of vitamin A-deficient, control and stock colony rats has been examined by chromatographic and spectroscopic techniques.
2. Attention has been focused on the spectroscopically recognizable constituents designated SA and SC, together with total sterol and 7-dehydro steroid; the concentration of all these substances has been shown to be affected by vitamin A deficiency.
3. In livers and submaxillary glands deficiency causes a rise in the level of SA; livers also exhibit a marked concentration of SC and there is a much smaller accumulation of this substance in the small intestine. The bladder, submaxillary gland and vagina contain an increased concentration of 7-dehydro steroid, and in the latter two organs and the testes there is accumulation of spectroscopically transparent sterol; the small intestine exhibits a marked fall in 7-dehydro steroid concentration.
4. These results are discussed in relation to the systemic mode of action of vitamin A.

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REFERENCES

- Festenstien, G. N., Heaton, F. W., Lowe, J. S. & Morton, R. A. (1955). *Biochem. J.* **59**, 558.
Feyel Cabanes, T. (1949). *C.R. Soc. Biol., Paris*, **143**, 1454.
Glover, M., Glover, J. & Morton, R. A. (1952). *Biochem. J.* **51**, 1.
Glover, J., Goodwin, T. W. & Morton, R. A. (1947). *Biochem. J.* **41**, 97.
Green, H. N. & Mellanby, E. (1928). *Brit. med. J.* **2**, 691.
Heaton, F. W., Lowe, J. S. & Morton, R. A. (1955). *Biochem. J.* **60**, xviii.
Lowe, J. S., Morton, R. A., Cunningham, N. F. & Vernon, J. (1957). *Biochem. J.* **67**, 215.
Lowe, J. S., Morton, R. A. & Harrison, R. G. (1953). *Nature, Lond.*, **172**, 716.
McCollum, E. V. & Davis, M. (1913). *J. biol. Chem.* **15**, 167.
Mayer, J. & Krehl, W. A. (1948). *J. Nutr.* **35**, 523.
Monaghan, B. R. (1932). *J. biol. Chem.* **98**, 21.
Morton, R. A. & Rosen, D. G. (1949). *Biochem. J.* **45**, 612.
Osborne, T. B. & Mendel, L. B. (1913a). *J. biol. Chem.* **15**, 311.
Osborne, T. B. & Mendel, L. B. (1913b). *J. biol. Chem.* **15**, 317.
Ward, R. J. & Moore, T. (1953). *Biochem. J.* **55**, 295.

Vitamin A Deficiency in the Domestic Fowl

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The observation that vitamin A-deficient rats accumulate two substances, SA and SC, in the liver (Lowe, Morton & Harrison, 1953; Heaton, Lowe & Morton, 1955, 1957) makes it necessary to ascertain by experiment whether the phenomenon is shown by other species as part of the deficiency syndrome.

The present paper describes experiments in which (a) very young chicks were placed on a vitamin A-deficient diet and compared with controls, and (b) fowls 16 weeks old (equal numbers of cockerels and pullets) were divided into two groups, one of which was given vitamin A in known amount as a supplement to the diet which in the other group resulted in avitaminosis A after about 8 weeks.

EXPERIMENTAL

Young chickens

Males of the Light Sussex breed were used. In a trial run they were a week old when received and put on the experimental diet. In the main experiment 1-day-old birds were given a commercial 'starter' mash for 1 week and then were put on the special diets.

Housing. The chickens were housed in an oval-shaped run with sheet-metal sides 3 ft. high. The concrete floor was covered to a depth of 4 in. with peat and the run was closed at the top with $\frac{1}{2}$ in. mesh wire netting. The temperature just above the peat was regulated by means of an infrared lamp (150w) suspended at a variable height over the run. The distance from the lamp to the chickens was increased from week to week so that the temperature was lowered from 90° to 70° F by 5°/week. After 5 weeks there was no further need to use the infrared lamp as the temperature of the

animal house was kept at 70° F by thermostatically controlled electric heaters.

The trial run established that the experimental diet produced avitaminosis A, and that no trace of vitamin A could be found in the livers of the chickens when they were killed.

In the main experiment fourteen male chickens were used. A vertical partition was placed across the run and two infrared lamps were placed so that each half of the run could be warmed. Seven chickens were placed in each compartment and water was provided at all times. The birds were weighed regularly.

Diet. Table 1 gives the composition of the diet supplied to the chicks. It is based on the work of J. G. Bieri (personal communication) and the salt mixture on that of Briggs, Spivey, Keresztesy & Silverman (1952). It should be noted that after 2 weeks the cerelose was replaced without detriment by sucrose. The control chickens were given vitamin A to the extent of 3 i.u./g. of diet. The amount of food supplied was 20 g. daily per chick for the first week, increased to 25 g. in the second week and so on, until in the sixth week the chickens were offered 40 g. per day each.

Older birds

Chicks of the Brown Leghorn breed which had been reared on free range were brought into the animal house at the age of 6 weeks and at first were housed three to a large cage and later in separate cages. After 10 weeks in the laboratory the birds were divided into two groups of twelve. Each group contained six males and six females.

Diets. From the age of 6–16 weeks the fowls were given diet A (Table 2). This provided vitamin A sufficient to meet the estimated normal requirement but not enough to build up a big reserve in the liver. Vitamin A was withheld from half the birds from the seventeenth week. This group