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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

received an extra 1% of limestone flour but had a negligible intake of carotenoids and of vitamin A. Towards the end of the experiment the deprived fowls were not eating all the food offered. Water was provided freely.

At the end of the feeding trials, when all the vitamin A-deficient birds had been killed, the controls were without exception healthy, and the pullets were laying eggs regularly but the yolks were colourless. It was then decided to give the controls for a short time a normal food mixture which contained carotenoids. The fowls quickly stored xanthophylls, the pullets produced eggs with yellow yolks and xanthophylls were deposited in the usual sites in both sexes.

Table 1. *Vitamin A-deficient diet*

Components are given in the order of mixing.

Salt mixture*	71 g.
Marmite	50 g.
Vitamin solution†	20 ml.
Arachis oil	40 g.
(containing vitamin K ₁	1 mg.
α-tocopheryl acetate	10 mg.
and vitamin D ₃ , 100 000 i.u./g., premixed)	30 mg.
Cerelose‡	600 g.
Light White Casein (Genatosan)	280 g.

* Salt mixture (Briggs *et al.* 1952): CaCO₃, 75 g.; K₂HPO₄, 45 g.; Na₂HPO₄·12H₂O, 92 g.; Ca₃(PO₄)₂, precipitated, 70 g.; NaCl, 44 g.; MgSO₄·7H₂O, 25 g.; ferric citrate, 2 g.; MnSO₄·4H₂O, 2.1 g.; KI, 0.2 g.; ZnCO₃, 0.1 g.; CuSO₄·5H₂O, 0.1 g. This is enough for five batches of the major components.

† Vitamin solution: thiamine hydrochloride, 32.5 mg.; riboflavin, 25 mg.; Ca pantothenate, 85 mg.; nicotinamide, 350 mg.; pyridoxine hydrochloride, 30 mg.; biotin, 1.25 mg.; *p*-aminobenzoic acid, 100 mg.

‡ After 2 weeks the cerelose was replaced by sucrose.

Table 2. *Diet A*

The diet was fed in the laboratory to 24 chickens until they reached the age of 16 weeks, and thereafter was fed minus vitamin A to twelve chickens until they became deficient. The twelve controls continued to receive this diet until all the deficient group had been killed, and were then transferred for a short time to a normal ration containing carotenoids. Amounts used were 50 g./day in the seventh week, increasing by 10 g./day in each succeeding week, until in the seventeenth and subsequent weeks the amount was 120 g./day.

	%
Dried skimmed milk	2.5
Dried whey	2.5
Extracted sunflower-seed meal	5.0
Extracted soya-bean meal	5.0
Pollards	28.75
Wheat	15.0
Milo	35.0
Dried yeast	2.5
Minerals*	3.75
Vitamin D ₃ , 2.2 i.u./g.	
Vitamin A, 3 i.u./g.	

* NaCl, 10; Ca₃HPO₄, 45; CaCO₃ (limestone flour), 40; trace-element limestone, 5%.

Avitaminosis

The deficiency symptoms observed are described below. It was planned to kill the birds when deficiency had become evident; in general this aim was achieved with the older birds, but some of the young birds died suddenly, with only early signs of depletion and no interruption of growth.

Examination of tissues

After the birds had been killed and the required tissues had been dissected out, the procedure described by Heaton *et al.* (1957) was followed. The tissues (if necessary stored at -20°C until worked up) were saponified and the unsaponifiable fractions chromatographed on alumina (P. Spence and Co., Widnes; Grade O weakened with water to Brockmann Grade 3).

RESULTS

Deficiency symptoms in young chicks

In the trial experiment the earliest sign of deficiency appeared after 18 days. One bird had difficulty in standing and was easily knocked over by the others. Three days later another chick developed similar weakness which was shown in turn by all the birds. Even when squatting the birds were unsteady and liable to fall down; there was progressive weakness but little or no loss of appetite or weight. Between the first signs of unsteadiness and death there was an interval of about 6 days.

Post-mortem examinations showed that all but one of the chicks had pale mottled kidneys with deposits of urates in the tubules.

In the main experiment unsteady gait first appeared after 20 days of vitamin A deprivation, and within a week the seven chicks in the group had died or had been killed. None of the control chicks showed any abnormality. The vitamin A-deficient chicks increased in weight steadily, indeed almost as well as the controls. They showed no signs of xerophthalmia or of lesions in the trachea or oesophagus. The ataxia described was the only symptom of deficiency in the living bird, and kidney damage was the only post-mortem sign.

Deficiency symptoms in older fowls

The birds in this experiment were hatched on 25 April 1955, and put into cages on 7 June. In mid-July some of the cockerels were becoming definitely bow-legged but the condition was shown by X-ray examination not to be a rachitic one, nor was it due to manganese deficiency. Our veterinary colleagues were inclined to regard the cause as environmental rather than dietetic. The fowls were divided into groups on 16 August and the first sign of deficiency was recorded on 3 October. 'One of the cockerels made a gurgling sound, flapped its wings a little and fell to the bottom of the cage as though dead. After a short time, probably 30 sec.,

it seemed to recover and within 5 min. it was eating again. At first the legs seemed weak but ability to stand normally soon returned.' A few days later other cockerels were similarly affected and indeed one or more of these bouts was witnessed in each of the deprived cockerels. There always seemed to be difficulty in breathing, possibly because of blockage in the respiratory tract. Not a single pullet was seen to suffer anything resembling these fits of unconsciousness.

The females showed marked leg weakness and were often found sitting or leaning against the side of the cage. They would also use a wing to prop themselves upright [see Fig. 1 (a), (b)]. The females began to show this ataxia from 7 October onwards. Each pullet was killed when it became unsteady on its feet and lost interest in its food. Anorexia was a general but late symptom. (One hen was found to have 'big liver disease', a form of leukaemia. This is regarded on veterinary advice as an incident unconnected with vitamin A deficiency and irrelevant to the present inquiry.) In general, the vitamin A-deficient birds exhibited white patches in the

trachea, and subsequent microscopic examination showed the presence of many squamous epithelial cells in the slime. Pustules in the oesophagus appeared as a late manifestation, mostly in males. When seen they were very marked [see Fig. 1 (c)]. Mucus in the nasal sinuses was frequently observed. Degeneration of the nictitating membrane with accumulation of cheesy material beneath the eyelids sometimes occurred as an almost terminal symptom of deficiency.

The male control birds showed normal comb growth and the testes were well developed, but the deprived cockerels showed very small combs and underdeveloped testes [Fig. 1 (d)]. Similarly, the ovaries of the deprived hens were small and underdeveloped. The control hens were laying regularly from 31 October onwards but no eggs were laid by the vitamin A-deficient pullets. On post-mortem examination most of the deficient birds had abnormal kidneys, and it seemed that the kidney lesions were more severe in the few birds which died than in those which were killed. Table 3 summarizes the symptoms observed.

Fig. 1. (a) Vitamin A-deficient cockerel in a temporary fit of unconsciousness. (b) Vitamin A-deficient pullet unable to stand. (c) Oesophagus from adult vitamin A-deficient cockerel. The organ was slit lengthwise and laid flat. The white spots are pustules. (d) The cockerel on the right was given vitamin A and shows a normal comb. The cockerel on the left was vitamin A-deficient and shows a much smaller comb.

Blood analysis for uric acid

Blood was taken from the wing veins of control and vitamin A-deficient birds just before they were killed (12 controls, 8 vitamin A-deficient birds). The

uric acid content was determined by means of Folin's (1934) reagent (King, 1946). The average value was 4.5 mg./100 ml. and no significant difference was obtained between the control birds and the deficient birds for either sex.

Table 3. *Symptoms observed in fowls deprived of vitamin A*

Symptom	No. of birds affected out of 6	
	Males	Females
Loss of appetite	6	6
Bouts of unconsciousness accompanied by difficulty in breathing	6	0
Failure to stand (leg weakness)	2	5
Lesions of the external parts of the eye; degeneration of nictitating membrane	3	2
Underdeveloped sex organs	6	6
Mucus blocking nasal sinuses	3	2
Oesophageal pustules	5	3
Tracheal lesions	6	4
Kidney lesions	Most severe in birds that died, but present in all	

Examination of tissues

Tables 4 and 5 summarize for chicks and fowls respectively the weights of tissue, the percentages of unsaponifiable matter and the gross spectral absorption for the control and the deficient groups. In the case of liver tissue the rather large amounts of vitamin A shown by the control groups made chromatography essential before the amounts of other constituents could be estimated. Indeed examination of the unfractionated unsaponifiable fractions for all the tissues is mainly useful in confirming that the deprived birds were in fact very thoroughly depleted of vitamin A.

Chromatographic analyses (Tables 6 and 7). Fractionation of the liver unsaponifiable matter from the controls showed that: (i) light petroleum eluted a small fraction containing a '260 m μ '

Table 4. *Unsaponifiable matter from chicken tissues*

Description	Average wt. of organ (g.)	Unsaponifiable matter (%)	Absorption spectrum in cyclohexane (m μ)	$E_{1\text{ cm.}}^{1\%}$
(a) Liver				
Vitamin A-deficient (trial)	11.4	0.47	$\lambda_{\text{max.}}$ 272 ~ 277 ~ 330	4.4 4.1 0.8
Vitamin A-deficient	12.3	0.35	$\lambda_{\text{max.}}$ 272 ~ 276 ~ 330	3.6 3.5 0.7
Control	12.7	0.33	$\lambda_{\text{max.}}$ 272 ~ 276 ~ 320 $\lambda_{\text{max.}}$ 326	8.2 8.1 3.3 3.4
(b) Kidney				
Deficient (trial)	3.4	0.56	$\lambda_{\text{max.}}$ 270 ~ 278	2.0 1.9
Deficient	4.2	0.45	~ 270 ~ 278	1.5 1.4
Control	4.7	0.27	$\lambda_{\text{max.}}$ 271 ~ 275 ~ 320	2.8 2.7 0.8
(c) Intestine				
Deficient (trial)	12.0	0.34	$\lambda_{\text{max.}}$ 271 $\lambda_{\text{max.}}$ 280	2.2 2.2
Deficient	11.3	0.30	$\lambda_{\text{max.}}$ 270 ~ 275 ~ 330	2.1 1.9 0.4
Control	14.04	0.31	~ 263 $\lambda_{\text{max.}}$ 270 ~ 280 ~ 293 ~ 330	2.6 2.9 2.7 1.6 0.7

~ = inflexion.

Table 5. *Unsaponifiable matter from fowl livers, intestines, kidneys and other tissues*

Description	Average wt. of organ (g.)	Unsaponifiable matter (%) [*]	Absorption spectrum in cyclohexane (m μ)	E ₁ % cm.
<i>(a) Liver</i>				
Control cockerels	41.6	0.358	$\lambda_{\max.}$ 327 $\lambda_{\max.}$ 255	34.8 9.1
Control pullets	44.4	0.364	$\lambda_{\max.}$ 327 $\lambda_{\max.}$ 257	21.3 7.8
Deficient cockerels	22.6	0.388	† $\lambda_{\max.}$ 272 277	2.9 —
Deficient hens	26.2	0.444	† $\lambda_{\max.}$ 272 277	1.95 1.7
<i>(b) Kidney</i>				
Control cockerels	16.7	0.27	$\lambda_{\max.}$ 272 320	2.53 1.55
Control pullets	17.5	0.32	$\lambda_{\max.}$ 272 320	2.74 0.88
Deficient cockerels	12.5	0.25	† $\lambda_{\max.}$ 272 277 330	2.02 1.95 0.47
Deficient hens	9.6	0.42	† $\lambda_{\max.}$ 272 280	1.34 1.26
<i>(c) Intestine</i>				
Control cockerels	35.6	0.29	† $\lambda_{\max.}$ 272 325	2.5 0.9
Control pullets	26.5	0.25	† $\lambda_{\max.}$ 272 320	3.1 0.76
Deficient cockerels	37.6	0.33	$\lambda_{\max.}$ 272	2.5
Deficient pullets	31.8	0.31	$\lambda_{\max.}$ 272 282	1.5 1.4
<i>(d) Oesophagus and crop</i>				
Control cockerels	16.9	0.25	270 325	1.56 0.57
Control pullets	13.2	0.24	270 340	1.22 0.32
Deficient cockerels	15.0	0.21	270 280	1.4 1.23
Deficient pullets	15.8	0.30	270 280 330	1.25 1.2 0.4
<i>(e) Gizzard</i>				
Control cockerels	31.8	0.18	† $\lambda_{\max.}$ 268 320	2.0 0.5
Control pullets	18.5	0.19	† $\lambda_{\max.}$ 270 330	2.7 0.5
Deficient cockerels	28.3	0.24	$\lambda_{\max.}$ 269	4.9
Deficient pullets	22.0	0.20	$\lambda_{\max.}$ 270	1.8
<i>(f) Trachea</i>				
Control cockerels	5.1	0.27	$\lambda_{\max.}$ 270 282 325	2.3 2.3 1.0
Control pullets	3.7	0.28	$\lambda_{\max.}$ 170 280	1.9 1.8
Deficient cockerels	5.7	0.19	$\lambda_{\max.}$ 271 281 330	1.9 1.8 0.9
Deficient pullets	3.0	0.29	$\lambda_{\max.}$ 272 277 340	2.2 2.1 0.7

^{*} Based on the wt. of the organ.

† Carotenoids, mainly carotenols, were also present.

† No colour with SbCl₃ reagent for vitamin A.

substance, anhydrovitamin A and carotenoid hydrocarbon; (ii) 2 % ether-light petroleum eluted further carotenoid products; (iii) 4 % ether-light petroleum began to remove vitamin A which, however, in the main accompanied the cholesterol, which was eluted by 8-20 % ether-light petroleum.

Ether alone eluted a mixture of xanthophylls accompanied by what might have been a little tocopherol. Ethanol eluted further xanthophylls.

The bulky sterol-vitamin A fraction was acetylated and the mixed acetates were chromatographed again on alumina (Brockmann, Grade 3). The bulk

Table 6. *Comparison after chromatography of unsaponifiable fractions obtained from tissues of vitamin A-deficient chicks and controls given vitamin A*

Description	Vitamin A (i.u./g. of tissue)	SA (u./g. of tissue)	SC (u./g. of tissue)	Total sterol (mg./g. of tissue)	7-Dehydro steroid (µg./g. of tissue)	Carotene (µg./g. of tissue)	Xanthophyll (µg./g. of tissue)
(a) Liver							
Deficient (trial)	a	10.8	a	3.0		Not estimated	
Control	14.7	6.98	0.36	2.2	0.78	0.05	0.32
Deficient	a	5.18	0.38	2.7	0.70	a	0.21
(b) Kidney							
Deficient (trial)	a	1.68	a?	3.4	Trace		Very low
Control	3.8	1.91	a?	1.9	Trace		Very low
Deficient	a	—	a?	2.6	Trace		Very low
(c) Intestine							
Deficient (trial)	a	0.52	0.16	2.0	0.6	a	Not estimated
Control	a	1.57	0.16	2.2	6.0	a	0.19
Deficient	a	2.04	0.16	2.3	0.5	a	0.04

a signifies not detected.

Table 7. *Comparison after chromatography of unsaponifiable fractions obtained from tissues of vitamin A-deficient fowls and controls given vitamin A*

Description	Vitamin A (i.u./g. of tissue)	SA (units/g. of tissue)	SC (units/g. of tissue)	Steroid (%)	'Dienone' (mg./100 g.)
(a) Liver*					
Control cockerels	205	6	1.1	0.25	a
Control pullets	122	9.1	0.9	0.24	a
Deficient cockerels	a	2.4	0.8	0.27	0.04
Deficient pullets	a	7.1	0.7	0.34	0.04
(b) Kidney*					
Control cockerels	7.8	1.2	0.7	0.18	a
Control hens	5.0	2.8	1.2	0.18	0.03
Deficient cockerels	a	1.5	0.3	0.21	a
Deficient pullets	a	1.4	0.35	0.30	0.02
(c) Intestine†					
Control cockerels	a	0.94	0.46	0.19	0.03
Control pullets	a	0.32	0.28	0.17	0.034
Deficient cockerels	a	0.25	a	0.21	0.023
Deficient pullets	a	0.28	a	0.24	0.019
(d) Oesophagus and crop					
Control cockerels	a	0.3	a	0.146	a
Control pullets	a	a	a	0.136	0.07
Deficient cockerels	a	a	a	0.16	0.09
Deficient pullets	a	a	a	0.19	a
(e) Gizzard					
Control cockerels	a	0.7	a	0.29	0.18
Control pullets	a	0.9	a	0.13	0.12
Deficient cockerels	a	0.3	a	0.17	0.90
Deficient pullets	a	0.6	a	0.13	0.11

a = not detected.

* Where two batches were studied, the results have been expressed as means. Because of the nature of the separations the values given for SA and SC are only approximate.

† Also contained 0.3-0.9 mg./100 g. of 7-dehydro steroid.

Table 8. *Further examination of gizzard tissues from normal healthy cockerels*

Gizzard tissue (a)	Wt. of tissue (g.)	Unsaponifiable matter (%)	Absorption spectrum in cyclohexane (m μ)	E ₁ ¹ % _{cm.}	Cholesta-3:5-dien-7-one (mg./100 g. of tissue)
Lining	26.5	0.224	267	4.63	0.05
Muscle	275.0	0.21	λ_{max} . 270	1.36	0.025
(b)		Percentage lipid			
Lining	35	0.24	230 260 320 λ_{max} . { 412 436 470	19.9 10.8 4.8 12.6 5.0 2.2	— — None found — — None found
Muscle	79	1.97	λ_{max} . 270 280 400	1.0 0.9 0.1	— — —

(c) Gizzards were separated into lining and muscle and lipid was extracted from each tissue. The residue was in each case digested with alkali and the unsaponifiable fraction extracted.

Tissue	Fraction tested	Cholesta-3:5-dien-7-one (mg./100 g. of tissue)
Gizzard lining	Unsaponifiable fraction from lipid	None found
	Unsaponifiable fraction from residue	0.03
Gizzard muscle	Unsaponifiable fraction from lipid	None found
	Unsaponifiable fraction from residue	0.01

of the product, i.e. cholesteryl acetate and vitamin A acetate, was eluted easily by 1% ether-light petroleum. Fractions eluted by more polar mixtures indicated that SC-acetate was present together with carotenoids. In order to make certain that SC was present the fractions showing λ_{max} . 275 m μ were combined, dissolved in a little light petroleum and cooled by means of a mixture of ice and salt. An ice-cold solution of iodine in petroleum was then added with vigorous stirring. After 10 min. the mixture was filtered and excess of iodine removed from the filtrate by washing with aqueous sodium thiosulphate. The precipitate, consisting of a carotenoid-iodine adduct, was discarded. The light-petroleum solution, after washing and drying, displayed the spectrum of SC-acetate very clearly. The iodine method of removing carotenoids was first used by Kuhn & Lederer (1932).

The results (Table 7) on gizzard indicated the presence of cholesta-3:5-dien-7-one in relatively high quantity. It was decided to follow up this observation by examining the muscle wall and the lining separately (Table 8). There was less dienone in the gizzards of normal cockerels than was found in those of the birds on the experimental diet, but the substance was detected after chromatography and differentiated from SA by its selective absorption at 355 m μ in concentrated sulphuric acid.

Weight for weight the lining contained twice as much dienone as muscle, but no cholestadienone could be found in the lipid from either tissue. If, however, the residue which remained after the comminuted tissue had been exhaustively extracted with hot ethanol was itself digested with alkali and refluxed in the presence of ethanol, the ether extract contained the dienone. From these experiments it would seem that cholesta-3:5-dien-7-one (or a precursor) is present in the non-lipid portion of both the linings and the cell wall. Leg muscle of fowl saponified similarly did not yield any dienone.

DISCUSSION

The earlier work on vitamin A deficiency in young chicks (Hart, Halpin & Steenbock, 1922; Emmett & Peacock, 1923; Elvehjem & Neu, 1932; Almquist & Mecchi, 1939) showed that ataxia, inco-ordination and ruffled feathers appeared after about 4 weeks without vitamin A; there was little evidence of xerophthalmia or loss of appetite. Kidneys were pale and the tubules distended by accumulations of urates. Cruickshank, Hart & Halpin (1927) suggested that as they found only slight kidney lesions in chicks expected to survive only a day or so, the accumulation of urates in renal tubules and ureters was a late and possibly somewhat indirect sequel to vitamin A depletion.

Inflammation about the eyelids has been seen in older deprived birds (Taylor, Stern, Russell & Jungherr, 1947); but it is widely agreed that the xerophthalmia, which is a reliable indicator of avitaminosis A in rats, is not seen in young chicks. On the other hand, the leg weakness and uncertain gait of depleted chicks is a good indicator and is distinct from rickets.

The keratinizing metaplasia of vitamin A deficiency (Wolbach, 1954) is sometimes shown in the mouth, larynx, pharynx and oesophageal glands of vitamin A-deficient fowls (Almquist & Mecchi, 1939), but the lesions are more marked in older birds than in young chicks. Fletcher & Rigdon (1949) recorded well-defined pathological changes in the central nervous system of vitamin A-deficient ducks, and Rigdon (1952) found conspicuous degeneration of nerve cells not attributable to mechanical pressure by deformed bone on nerve. The view (cf. Rude, Bieri & Rigdon, 1951) that in vitamin A-deficient chicks bone distortion cannot account for the neurological symptoms is not consistent with the experience of Wolbach, Bessey & Hegsted (1952) and with the work of Mellanby (1938, 1947) on mammals. Adamstone (1947), on the other hand, found in vitamin A-deficient chicks slow growth of both skeleton and central nervous system.

Lamming, Woollam & Millen (1954), however, reported in vitamin A deficiency a stenosis of the cerebral aqueduct and hydrocephalus. Woollam & Millen (1955) observed as an early sign of vitamin A deficiency in the chick a significant rise in cerebrospinal-fluid pressure.

There are neurological sequelae to vitamin A deficiency, but the nature of the primary dysfunction has yet to be established. If a serious decrease in blood plasma vitamin A level has as its primary morphological sequel a thickening of the cerebral aqueduct the resulting stenosis could raise the cerebrospinal fluid pressure. The pathology of vitamin A deficiency might then vary from one species to another, in the sense that comparative anatomy would determine which neurological manifestation would be the first to appear in the different species.

The deficiency syndrome in young chicks described on p. 216 reproduced the experience of earlier workers. Chromatography carried out on the unsaponifiable fractions from liver and kidney showed that vitamin A was absent from these organs in the deprived animals and present in the controls. SA was found in all the tissues studied (Table 6), but the differences between the controls and the deprived chicks were not appreciable. SC was detected in liver and intestine but doubtfully in kidney. The only substantial difference between the vitamin A-deficient and the control group was in

respect of the 7-dehydro steroid content of the tissue of the small intestine. This fell in deficiency to one-twelfth of that shown by the controls. A similar effect was also seen in rats (Heaton *et al.* 1957).

The portion of liver unsaponifiable matter most readily eluted by non-polar or weakly polar eluents probably consists mainly of transparent hydrocarbon contaminated with a substance showing $\lambda_{\max}$ 260 m μ . The amount of this material seems to increase as a result of vitamin A deficiency both in chick and rat.

For SA and SC, however, the chick experiments and the rat experiments are quite different. In rat liver SA rises and SC rises greatly in avitaminosis A, whereas in chick liver no change occurs.

In the experiment with older birds the chicks were given a good start on free range and from the age of 6–16 weeks all the birds received 3 i.u./g. of vitamin A in their food. This amount was sufficient to meet the requirement (Williams, Lampman & Bolin, 1939; Sherwood & Fraps, 1940; Jungherr, 1943) and in the controls provided for normal growth and development, including production of eggs. No vitamin A was found in liver or kidney in the deprived birds and the reserves had been exhausted before the birds were killed or died. The bouts of unconsciousness suffered by the cockerels do not seem to have been described before and the marked sex difference is new. The gross differences in the appearance of secondary sex characteristics are important and afford strong evidence of an influence, possibly indirect, of vitamin A on sterol metabolism.

Although vitamin A deficiency was shown in the deprived cockerels and pullets, no substantial difference in respect of SA, SC, total sterol, 7-dehydro sterol and cholesta-3:5-dien-7-one could be found (Table 7) between the tissues of the control and the deficient birds. The gizzard may be an exception but this requires further study.

For the adult birds, as for the chicks, there is an unequivocal failure to reproduce the changes in SA- and SC-content shown by the rat, although these two substances are regularly found in small amounts in the organs of both chicks and older birds.

The work on the rat (Lowe *et al.* 1953; Heaton *et al.* 1957) envisaged the possibility that SA or SC (or both) accumulated because a metabolic process in which vitamin A was needed could no longer occur. The work on poultry does not support this conclusion. In the birds studied here SA and SC occur in amounts consistent with their being essential metabolites independent of vitamin A. This raises a problem concerning the origin of SA and SC in the rat, and it becomes necessary to inquire whether the microbiological situation in the alimentary tract is more sensitive in some species to vitamin A deficiency than in others.

SUMMARY

1. Young male chicks (Light Sussex breed) placed on a vitamin A-deficient diet became unsteady on their legs in the fourth week and soon afterwards died. The kidneys were pale and showed deposits of urates. There was, however, no loss of appetite or weight, no xerophthalmia or lesions in the trachea or oesophagus.

2. Older birds (Brown Leghorn breed) of both sexes were put on a vitamin A-deficient diet at 16 weeks of age. Control birds were given vitamin A as a supplement. Deficiency symptoms began to appear after 7 weeks of deprivation; the males suffered recurring brief loss of consciousness and the females found difficulty in standing. Epithelial lesions were a late symptom. Sexual development was greatly retarded in both sexes.

3. Various tissues (liver, kidney, intestine) were obtained from the young chicks and from control birds and the unsaponifiable fractions were examined by chromatographic and spectrophotometric methods. The rise in SA and SC which occurs in vitamin A-deficient rat tissue did not appear in the chick tissues.

4. Similar study of tissues (liver, kidney, intestine, gizzard, oesophagus and crop, trachea) from older birds of both sexes made vitamin A deficient revealed no real difference in respect of SA, SC, total sterol, 7-dehydrocholesterol and cholesta-3:5-dien-7-one between the controls and the deprived birds.

5. Preliminary work on gizzard muscle and lining showed the presence of cholesta-3:5-dien-7-one in both tissues.

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Vitamin A Deficiency in the Rat, With and Without Added Dietary Cholesterol

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Certain aspects of the vitamin A-deficiency syndrome in the rat have been studied by Heaton, Lowe & Morton (1955, 1957). These workers examined the unsaponifiable portions from various tissues of normal, control and vitamin A-deficient rats and drew attention to marked increases in two unidentified substances, designated SA and SC, in

the livers of the vitamin A-deficient rats. Lowe, Morton & Harrison (1953) had earlier put forward, as a working hypothesis, the idea that vitamin A exerts a restraining influence on the dehydrogenation of cholesterol or steroid hormones, such restraint being lifted in vitamin A deficiency. The hypothesis that this might account for the appearance of SA