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The protective action of vitamin A upon various tissues in the avitaminotic rat, and the sensitivity of these tissues to vitamin A deficiency

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The experiments reported here were undertaken to compare the sensitivity of some rat tissues to relative or absolute vitamin A deficiency and to prophylactic doses of the vitamin. That there must be considerable differences in the requirements of various tissues and processes in the rat for vitamin A is suggested by the computed requirements of the entire animal found in the literature, based on such phenomena as growth, tooth colour, vaginal smears, storage, and longevity, recently summarized by Rubin & De Ritter (1954). The figures given range from 10 to 1000 i.u./kg bodyweight/day.

In the present experiments the changes compared were those that occur in the incisor teeth (Wolbach & Howe, 1933), the medulla oblongata (Irving & Richards, 1938–9), the maxillary fundic alveolar bone (Irving, 1949) and the nasolacrimal duct (Irving, Pindborg, Fitzhugh, Weinmann & Schour, 1952). These were chosen as examples of two hard tissues, epithelia and nerve tissue. A preliminary account of some of these findings has already been published (Irving & Richards, 1954).

EXPERIMENTAL

Management of rats

Young albino or hooded Lister rats were used. Their mothers were given stock biscuits till the young were 16 days old. Then, as in previous experiments (Irving & Richards, 1938–9), the mother was placed on the same vitamin A-free diet as previously

used, and the young also had access to it. The composition of the diet was as follows: caseinogen 18, wheat starch 50, cottonseed oil 15, salts (McCollum, no. 185) 5, dried yeast 18·3, lemon juice 5 parts, with a small amount of potassium-iodide solution and sufficient water to produce a stiff paste. Vitamin D was supplied as Radiostol (British Drug Houses, Ltd) (0·02 ml./rat twice weekly). The mother was removed from the cage and given stock diet with greens and whole milk two or three times daily. The young rats were weaned at 23 days of age and continued on the vitamin A-free diet. Positive control animals were treated in the same way, save that from weaning they were given daily about 20 i.u. of vitamin A from a concentrate diluted in arachis oil.

Treatment

Experiment 1. This experiment was carried out on thirty rats, fifteen males and fifteen females. The animals of each sex were divided into three sets of five litter-mate rats. One rat from each set was killed on the 28th, 35th, 42nd or 49th day after weaning, irrespective of its clinical condition. The fifth rat of each set, killed on the 49th day, acted as a positive control.

Experiment 2. This experiment was carried out on thirty-six rats, eighteen males and eighteen females. As in Exp. 1, rats of each sex were divided into three sets of from five to eight litter-mates, and each rat received from weaning $\frac{1}{3}$, $\frac{2}{3}$, 1, $1\frac{1}{2}$, 2 or 3 i.u. vitamin A daily. They were all killed 50 days after weaning.

Experiment 3. This experiment was conducted entirely with male rats, seventy-one in all. They were divided into sets of sixteen to nineteen animals. Litter-mate animals were given 2, 4, 8 or 16 i.u. vitamin A daily from weaning, and killed 100, 150, 200 or 250 days later.

Histological technique

Preparation of sections. The medullas were dissected out and stained by the Marchi method, as previously described (Irving & Richards, 1938–9). The anterior part of each skull was removed and decalcified and sections from the mid-longitudinal plane of the upper incisor teeth were stained with haematoxylin and eosin. These sections contained, besides the teeth, the alveolar fundic bone and the nasolacrimal duct, which runs under the concave side of the tooth, as described by Irving *et al.* (1952).

Assessment of the lesions. Four arbitrary stages, designated 1, 2, 3 and 4, were used to indicate slight, moderate, marked and extreme changes in the tissues examined. These corresponded to the stages used previously for the central nervous system (C.N.S.) and teeth by Irving & Richards (1938–9, 1939).

RESULTS

A brief description of the lesions encountered in each tissue will be given. In vitamin A deficiency a degenerative change is found in the medulla oblongata of the rat in a tract known as the funiculus praedorsalis, a motor tract corresponding to the tectospinal tract in man. The degeneration is maximal at the level of the pyramidal decussation; in these experiments, the medullas were examined at this level. In the incisor teeth, the earliest change is in the lingual odontoblasts, which first become greatly reduced

in height and then show irregular down-growths into the dentine. This change extends later to the labial odontoblasts and dentine. Subsequently there is a relative over-formation of dentine on the labial side, and finally the enamel organ undergoes a degenerative change. In the fundic bone, new bone of a primitive appearance is laid down on the side nearest the tooth, where normally resorption takes place. This new bone extends usually in tongue-shaped outgrowths into the periodontal tissues. The nasolacrimal duct is normally lined with pseudo-stratified ciliated columnar epithelium. In vitamin A deficiency this epithelium becomes stratified squamous epithelium; in a more advanced deficiency it becomes keratinized and the entire duct may be plugged by a keratinized mass.

Table 1. *Exp.* 1, *animals on the vitamin A-free diet. Comparative effects of vitamin A deficiency upon various tissues of the rat, shown by assigning arbitrary numbers to the degree of change—*0 *being no change and* 4 *the most severe degeneration or metaplasia or both*

		Days				
Tissue	Litter	28	35	42	49	49 (Positive controls)
			Males			
C.N.S.	N	3	3	2	2	0
	C	3	3	3	3	0
	K	2	3	4	4	0
Tooth	N	0	2	2	2	0
	C	1	2	2	2	0
	K	1	2	2	3	0
Fundic bone	N	0	1	2	2	0
	C	1–	0	3	3	0
	K	1–	1	2	3	0
Nasolacrimal duct	N	2	2	2	2	0
	C	2	2	2	2	0
	K	2	3	2	3	0
			Females			
C.N.S.	*a*	3	2	3	3	0
	b	2	3	3	3	0
	d	2	3	3	4	0
Tooth	*a*	0	2	2	2	0
	b	0	1	2	2	0
	d	0	1	2	2	0
Fundic bone	*a*	0	0	1	3	0
	b	0	0	1	1	0
	d	0	0	1	2	0
Nasolacrimal duct	*a*	2	3	4	2	0
	b	2	3	3	2	0
	d	1	2	2	2	0

Experiment 1. The results are shown in Table 1. At 28 days, the medulla already showed severe degeneration, whereas the tooth and fundic bone showed slight or no changes. The nasolacrimal duct showed metaplasia and keratinization of the epithelium. As the experiment continued up to 49 days the C.N.S. changes became more advanced

and the tooth and fundic-bone changes became progressively more severe. The changes in the nasolacrimal duct also became somewhat more marked. The tooth changes did not develop to an extreme degree, since the animals were killed before this could occur. As pointed out previously (Irving & Richards, 1939), animals on a vitamin A-free diet usually die before marked dental changes can be seen; such marked changes occur only if the animals are kept alive by suboptimal doses of vitamin A. It would thus appear that the C.N.S. and nasolacrimal ducts are more sensitive to vitamin A lack in the early stages of the deficiency than is the tooth or the fundic bone. As previously noted (Irving & Richards, 1938–9; Coetzee, 1949) females developed the lesions in the medulla later than males. The positive controls showed none of these pathological lesions.

Table 2. *Exp.* 2, *animals on the vitamin A-free diet, given for* 50 *days graded daily doses of vitamin A. Protective action of vitamin A upon various tissues of the rat, shown by assigning arbitrary numbers to the degree of change—*0 *being no change and* 4 *the most severe degeneration or metaplasia or both*

		Dose of vitamin A (i.u./day)						
Tissue	Litter	0	$\frac{1}{3}$	$\frac{2}{3}$	1	$1\frac{1}{2}$	2	3
					Males			
C.N.S.	G	3	3	0	0	0	0	—
	H	—	3	2	1	0	0	0
	L	—	3	2	0	0	0	0
Tooth	G	3	3	1	0	0	0	—
	H	—	3	1	0	0	0	0
	L	—	2	2	0	0	0	0
Fundic bone	G	1	1	1	0	0	0	—
	H	—	2	1	0	0	0	0
	L	—	1	2	0	0	0	0
Nasolacrimal duct	G	3	2	2	1	1	1	—
	H	—	3	1	1	1	1	0
	L	—	2	2	1	1	1	0
					Females			
C.N.S.	*u*	3	3	2	0	0	0	0 0*
	p	—	3	2	0	0	0	—
	s	—	3	2	0	0	0	—
Tooth	*u*	3	3	1	1	1	0	0 0*
	p	—	2	1	0	0	0	—
	s	—	3	1	0	0	0	—
Fundic bone	*u*	3	3	1	0	0	0	0 0*
	p	—	2	0	0	0	0	—
	s	—	2	0	0	0	0	—
Nasolacrimal duct	*u*	2	2	2	1	1	0	0 0*
	p	—	2	2	1	1	1	—
	s	—	3	2	1	1	0	—

* Two rats.

Experiment 2. The results are shown in Table 2. With the exception of the nasolacrimal duct, the C.N.S. of one animal and the teeth of two animals, all tissues of both males and females were protected for 50 days by 1 i.u. of vitamin A daily or more. The

fundic bone had apparently about the same vitamin A requirement as the tooth and C.N.S. The nasolacrimal duct, however, needed more vitamin A for normality, which was not attained till the dose level was 2–3 i.u. daily.

Table 3. *Exp.* 3. *Protective action of vitamin A upon various tissues of seventy-one male rats, shown by assigning arbitrary numbers to the degree of change—*0 *being no change and* 4 *the most severe degeneration or metaplasia or both*

Tissue	Degree of change (individual ratings) when dose given for period (days) 100	150	200	250
Dose: 2 i.u./day*				
C.N.S.	Only one rat, which had also very severely affected teeth, showed degeneration (stage 2 at 200 days)			
Teeth	4	2	4+	2
	2	3	4	3
	1	1	3	—
	1	3	1	—
	2	4	3	—
	0	—	—	—
	0	—	—	—
Fundic bone	0	0	2	0
	0	0	2	1
	0	0	0	—
	0	0	0	—
	0	1	0	—
	0	—	—	—
	0	—	—	—
Nasolacrimal duct	1	1	1	0
	1	1	1	1
	1	1	1	—
	1	1	1	—
	1	1	1	—
	1	—	—	—
	0	—	—	—

Tissue	Degree of change (individual ratings) when dose given for period (days) 100	150	200	250
Dose: 4 i.u./day†				
C.N.S.	All rats unaffected			
Teeth	0	0	0	1
	0	0	0	0
	0	1	1	0
	2	0	0	—
	—	0	—	—
Fundic bone	All rats unaffected			
Nasolacrimal duct	Only one rat showed degeneration (stage 1 at 250 days)			
Dose: 8 i.u./day‡				
C.N.S.	All rats unaffected			
Teeth	Only one rat showed degeneration (stage 1 at 200 days)			
Fundic bone	All rats unaffected			
Nasolacrimal duct	All rats unaffected			
Dose: 16 i.u./day§				
C.N.S.	All rats unaffected			
Teeth	0	0	0	1
	0	0	0	2
	0	0	0	0
	0	0	0	0
	—	0	0	—
	—	0	—	—
Fundic bone	All rats unaffected			
Nasolacrimal duct	All rats unaffected			

* Number of rats in group at 100, 150, 200 and 250 days: 7, 5, 5, 2.
† Number of rats in group at 100, 150, 200 and 250 days: 4, 5, 4, 3.
‡ Number of rats in group at 100, 150, 200 and 250 days: 4, 5, 4, 4.
§ Number of rats in group at 100, 150, 200 and 250 days: 4, 6, 5, 4.

At the end of this experiment the average weight of the rats was 115–120 g; thus for the protection of the C.N.S., tooth and fundic bone about 10 i.u. vitamin A/kg body-weight/day were adequate. To protect the nasolacrimal duct between 20 and 30 i.u./kg body-weight/day were required.

Experiment 3. The results are shown in Table 3. They were in general the reverse of those found in Exp. 1. Except in one animal, very severely affected at the 2 i.u.

level, the C.N.S. was completely protected up to 250 days by 2 i.u. or more of the vitamin daily. The tooth was not protected by 2 i.u. and became steadily worse with time. Better protection of the tooth was attained by 4 i.u. daily, but even with 8 or 16 i.u. daily some teeth in each group showed degenerative changes at 200 and 250 days. Most of the fundic bones were protected by 2 i.u. daily and all were normal on 4 i.u. and higher doses. The nasolacrimal duct almost uniformly showed metaplasia at the 2 i.u. level maintained over the period of the experiment. Virtually complete protection was afforded at the 4 i.u. level and complete protection at higher doses.

DISCUSSION

The results show that the relative sensitivity of various tissues to vitamin A lack and the degree of response to dosage with the vitamin are different at different ages. Thus, during the period of rapid growth, the C.N.S. and the epithelium of the nasolacrimal duct are severely affected, compared with the hard tissues studied. For the C.N.S. it was calculated from figures of Jackson (1913) and Donaldson (1924) that the growth of this tissue is very rapid in early life, and thus its requirement of vitamin A may be high. At 20 days of age, the brain of the rat has reached 60% of its final weight, whereas the liver and kidney do not reach this stage until the animal is over 50 days old. On the other hand, possible biochemical changes in the various tissues, which are different at different ages, may be the cause of these various sensitivities. All tissues are severely affected 50 days after weaning, but by this time the C.N.S., tooth and fundic bone have an equal protection. The level of requirement for protection of the tooth at this age agrees with that previously found (Irving & Richards, 1939), but the level for protection of the C.N.S. was slightly lower in the present study than previously reported (Irving & Richards, 1940). The nasolacrimal-duct epithelium is, however, more sensitive and requires about three times this dose of vitamin A for complete protection.

As the animal's growth slows down, the requirements of the different tissues change. The C.N.S. now is protected by a relatively small dose, but the tooth and the nasolacrimal-duct epithelium require at least twice as much of the vitamin. The fundic bone seems less sensitive. One animal, denoted 4+ in the tooth column of Table 3, which was on the 2 i.u. dose level for 200 days, had unusually severe signs of deficiency. It is of interest that it affected all its tissues, including the C.N.S., which in other animals had been protected. It is possible that in conditions of stress, the requirements of all tissues rise. The tooth results confirm what was previously reported by Irving & Richards (1939), that the daily requirements in absolute amounts of vitamin A for protection of this organ rise with age. The same applies to the nasolacrimal duct, but not to the fundic bone or C.N.S.; their requirements appear to be covered by, at most, 2 i.u. daily over a period of 150 days.

Other workers endeavouring to arrive at figures for the vitamin A requirement of the rat have compared the effects of different levels of intake upon various structures or processes. Thus Irving & Richards (1939, 1940) reported that at 7 weeks after weaning 1–1·5 i.u. vitamin A daily protected the medulla and incisor teeth from

degenerative changes and other parts of the body from pathological changes. Guilbert, Howell & Hart (1940) found an equal requirement for maintaining normal cornification of the vagina, normal growth and general well-being, but at this level of intake (18–22 i.u./kg body-weight/day) there was little or no storage of vitamin A in the liver. Lewis, Bodansky, Falk & McGuire (1942) concluded that, whereas 20 i.u./kg body-weight prevented deficiency signs, 250 i.u. were needed for optimal growth and 1000 i.u. for adequate storage in the liver. Moore (1943) reported that an intake of 6 i.u./day, though permitting almost as good growth as 16 i.u., did not restore the brown pigment of the incisor teeth of rats previously depleted of vitamin A. Campbell, Udiljak, Yarmolinsky & Sherman (1945) and Callison & Knowles (1945) likewise found that the lowest levels of vitamin A causing good growth gave negligible reserves in the liver. Paul & Paul (1946) stated that about the same dosage of the vitamin was needed for the protection of the eyes and teeth, for growth and for survival in rats (100 i.u./kg body-weight). The figure derived from our experiments for the protection of the nasolacrimal-duct epithelium at 50 days, 20–30 i.u./kg body-weight, is lower than that found by most other workers.

Many workers, noting that the requirement in absolute amount rises with age, have considered it to be a function of the body-weight. Coetzee (1949), however, pointed out that the ratio of body-weight of male and female rats bore no relation to the ratio of the vitamin required for protection of the C.N.S. in the two sexes. The present results, likewise, seem to indicate that the direct association of requirement with body-weight is too simple. The requirement is a function of that of the most sensitive tissue at the time of the experiment, and the most sensitive tissue is different at different ages. It is true that the C.N.S. is most sensitive when it is growing rapidly, the incisor tooth erupts continually and mucous membrane of the nasolacrimal duct is being continually replaced, whereas the metabolism of the fundic bone might be somewhat lower, but one cannot say that the activities of these tissues are related to body growth as a whole. It would appear that the only way to assess the true vitamin A requirement is to examine the whole body to find what level maintains all the tissues in an optimal condition. The differences found in the literature for the vitamin A requirement of the rat when many different structures or processes have been compared support this view.

SUMMARY

1. One hundred and thirty-seven rats were placed at 16 days of age on a vitamin A-free diet and were weaned at 23 days.

2. Thirty of these rats received the diet after weaning and were killed at intervals. The funiculus praedorsalis of the medulla oblongata and the epithelium of the nasolacrimal duct showed marked degenerative changes by 28 days after weaning, when the incisor tooth and maxillary fundic bone were normal or but little affected. By 49 days after weaning all these structures showed advanced changes.

3. Thirty-six rats were given from weaning doses of vitamin A, ranging from $\frac{1}{3}$ to 3 i.u. daily and were all killed 50 days later. The funiculus praedorsalis, incisor tooth and fundic bone were protected by 1 i.u. vitamin A daily, but the epithelium of the nasolacrimal duct was not protected until the dosage was 3 i.u. daily.

4. Seventy-one rats were given from weaning doses of vitamin A ranging from 2 to 16 i.u. daily, and were killed 100–250 days later. Over this period the funiculus praedorsalis was protected and the fundic bone was almost completely protected by 2 i.u. daily. The nasolacrimal-duct epithelium was protected by 4 i.u. daily, but the incisor tooth was totally unprotected by 2 i.u. daily, partly protected by 4 i.u., and not entirely protected even by 16 i.u.

5. We consider that the requirement for vitamin A is not a function of body-weight, but can only be based on protection of the tissue most sensitive to vitamin A deficiency. This tissue differs with age.

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