

## Depletion of Substantial Vitamin A - Reserves in Growing Rats.

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RATS require for normal growth, health and reproduction, a certain minimum level of vitamin A. This was found to be 18-22 International Units (I.U.) of vitamin A per Kgm. body weight, by Gross and Guilbert (1939). When rats receive vitamin A in excess of these levels, they are able to store vitamin A in the liver. Should the animals receive a ration deficient in this factor, the reserves are drawn upon to maintain normal growth, appetite and health.

In cattle, Riggs (1940) has found that the time required for total elimination of vitamin A from the livers was from 56 to 178 days, depending on the age of the animals (ages varying from 3 to 16 months) and also depending upon the levels of the reserves of vitamin A stored. Davies and Moore (1935, 1937) working with rats, came to the conclusion that when high reserves were built up due to massive doses of vitamin A given in the feed, the depletion is at first rapid but eventually the reserves reach a level when very small demands thereon are made, thus enabling the rats to reach maturity without suffering from a vitamin A deficiency.

In a later publication, Moore (1940) showed that vitamin E-deficiency in conjunction with vitamin A-deficiency, caused a more rapid elimination of the vitamin A-reserves. In the present work a ration was given which lacked both vitamin A and vitamin E and a study was initiated to determine the rate of vitamin A-elimination from the livers, when rats had previously stored about 1,200 I.U. of vitamin A. The ration was the same as the one used in other vitamin A-deficient work at this Institute (1943).

The percentage composition of the ration was:—

Modified Bacharach (1931) Ration	{	Casein (ether and alcohol extracted) ...	18.5 gm.
		Dextrinized Starch ...	50.0 gm.
		Fat ...	12.3 gm.
		Yeast ...	10.0 gm.
		Canesugar ...	0.08 gm.
		Salts (Kellermann—modified Steenbock)	5.12 gm.

The ration was freshly prepared each week and fed to the rats *ad lib.* The rats were divided into three groups: One group (Group A, control) was given the ration fortified with cod liver oil ( $\pm 2$  per cent.). An equal number of rats (16) with the same average body weight (*viz.* 103 grams)

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was fed the deficient ration (Group B). A third group (Group C) was also fed the deficient ration and was reserved for the time when livers would be depleted; in other words, the rats of this group would serve as examples of vitamin A-deficiency, apart from body weight. All the rats in these groups were males of nearly the same age (from 5 to 6 weeks old).

During the pre-experimental period the rats had access to a full, well-balanced ration (rat stock ration) as used by this Institute for general purposes of breeding, etc. During this time (up to 6 weeks) a reserve of about 1,200 International Units had been built up. (The stock ration contained cod liver oil as well as crushed yellow maize, which served as sources of vitamin A.)

Two rats were slaughtered in each group (A and B) at intervals of four weeks to obtain the levels of vitamin A in the livers by chemical method. The method of Moore (1930) was employed and the evaluation of the extracts was done by the Carr-Price test in the tintometer. The liver assays served as indication of the rate of depletion of vitamin A. All the rats were housed in a proper rathouse, well ventilated and heat controlled. Fresh feed and water were given *ad lib.* daily. At the stage when depletion was complete, attention was given to the third group of rats. Records of the symptoms of deficiency as they occurred were made.

CONCLUSIONS AND DISCUSSION.

It was seen that the depletion of the reserves in the deficient group was rapid (Table I). On the average there was a decrease of more than 50 per cent. of the total reserves within the first four to eight weeks. A less rapid rate of decrease followed in the next 8 weeks but by the 20th week the average liver reserves showed a decrease of 90 per cent. of the initial reserves. After 24 weeks the livers of the rats slaughtered were completely depleted of vitamin A and at the expiration of a further 4 weeks, at the 28th week, another set of rats were slaughtered and again the livers showed no vitamin A. It was thus conclusive that the livers were totally depleted. At the 26th week, symptoms of vitamin A deficiency were observed in the deficient group (the reserve group of rats). These symptoms are described in detail.

TABLE I.—Average Vitamin A Reserves of the Livers of Rats.

CONTROL GROUP.					VITAMIN A DEPLETION GROUP.				
Experimental Period.	Body Weight. Grams.	Liver Wht. Grams.	Whole Liver I.U. Vit. A.	Per gram. I.U. Vit. A.	Experimental Period.	Body Weight. Grams.	Liver Wht. Grams.	Whole Liver I.U. Vit. A.	Per Gram I.U. Vit. A.
Pre-experimental	109	7.0	1,120	162	Pre-experimental	101	5.5	1,123	145
After 4 weeks...	184	7.8	858	109	After 4 weeks...	165	8.5	424	52
After 8 weeks...	220	9.3	1,008	108	After 8 weeks...	207	9.5	504	53
After 12 weeks..	241	10.0	1,764	176	After 12 weeks..	228	8.0	319	40
After 16 weeks..	267	11.2	1,846	166	After 16 weeks..	241	8.4	263	31
After 20 weeks..	280	12.0	1,607	136	After 20 weeks..	275	11.2	124	11
After 24 weeks..	348	15.7	1,967	125	After 24 weeks..	268	10.4	0	0
After 28 weeks..	325	12.3	1,323	108	After 28 weeks..	276	7.8	0	0

In the control group the liver reserves showed after the first 4 weeks a decrease due mainly to a change in the diet. Thereafter an average increase in vitamin A reserves was noted for the following weeks, with a peak average value of 1967 International Units in the whole liver at the 24th week. At this stage the rats registered the highest body weights, whereas in the deficient group B the body weights of the individual rats had become constant at various earlier periods. The body weights were recorded at weekly intervals and the average body weight per group is given in Table 1, as well as the average liver reserves for the two groups.

From 20 to 24 weeks were necessary to deplete the liver reserves of growing rats fed a vitamin A-deficient ration when a liver reserve of 1,200 I.U. of vitamin A had been found in the initial stage. In another paper (1943) the author used younger rats, at weaning stage, with an average reserve of only 10 I.U. vitamin A. The depletion period of this small reserve was 43—52 days for two days for two sets of rats, intended for biological tests with vitamin A.

#### SYMPTOMS OF VITAMIN A-DEFICIENCY (GROUP C).

##### *(a) General Symptoms.*

At more or less the same time 3 rats of the Group C showed symptoms of vitamin A-deficiency. The early symptoms were observed in the eyes. Sensitivity towards light was obvious; the rats kept their eyes partly closed. The eyes on examination appeared dull compared with the controls. Dry, flaky discharge on the upper eyelids (keratinization) as well as deterioration of the eye glands were observed. Rats became progressively listless and lethargic, lying down most of the time, curled up with the head hidden from light. The appetite became poor and afterwards was completely lost. Food was left entirely untouched.

The symptoms following on the above were observed as the disease progressed, and can be considered as later, advanced symptoms. The mucous membrane of the lower jaw was tainted red and later bloody, especially the gums of the lower incisors. The mucous membrane of the nasal chambers was pale and dry, but later a catarrhal discharge, brown and sticky, was seen. Due to sensitivity towards extreme temperatures (heat) the breathing became laboured (not evident in control group) and in advanced stages, inhalations and exhalations were irregular with gasps or panting at intervals. The movement of the rats was generally affected due to degeneration of the muscles; at first difficulty in walking, crouching and dragging of the body were observed. Later the hind legs became partly paralysed (paresis) and the animals sprawled when resting; very often lameness in one limb or more was observed. The nervous system was also impaired. Rats twitched, shivered and shirked handling even though they were normally tame and used to handling. In extreme cases diarrhoea set in and in one case deafness was noted.

Schmidt (1941) described symptoms observed in ruminants, also Hart (1940) for cattle, horses, pigs and sheep, which are very similar to those seen by the author in the rats. Photographs are given as illustrations here.

(b) *Observations on Individual Rats in Group C.*

*Rat No. 1.*—Body weight 175 grams.

In one week after depletion the following successive symptoms were observed: The rat very gradually became worse and died soon afterwards; light sensitivity; a discharge of the eyes; loss of appetite and general listlessness; breathing became difficult with gasps at intervals; nasal discharge which irritated the animal considerably. On the last day, before death, the rat was very nervous, had convulsions of short duration (spasms). Diarrhoea had set in and the eyes appeared so badly affected that blindness was apparent. Body weight of rat (wasting had taken place rapidly) was 175 gm. compared with 296 gm. of one of the controls of the same age.

*Rat No. 2.*—Body weight 180 grams.

General symptoms as described were evident. The photograph clearly shows the rat in a sprawling attitude due to partial paralysis of the hind legs.

Curative treatment was practiced, at first by dosing small doses of fish liver oil (diluted down with olive oil to appropriate strengths). The dosing was done orally by pipette. As the rat improved on the third day vitaminized food was given. The appetite of the animal was quickly restored. The eyes became brighter and were soon apparently normal. Vitality improved. After ten days curative treatment the rat had gained 27 gm. in body weight. Hereafter the rat was fed the rat stock feed and gained 57 grams in weight 24 days after the treatment was started.

*Rat No. 3.*—Body weight 202 grams.

Symptoms as described above were observed, although they were in no way so severe as in the case of Rat No. 2 (see photographs). This rat could still walk with ease. The eyes, however, showed keratinization of the eyelids. The appetite was impaired and inactivity was evident.

After curative treatment the rat increased its bodyweight by 16 gm. in the first 10 days. In 24 days after the first treatment the rat was restored to perfect health and had gained in all 20 grams.

*Rat No. 4.*—Body weight 198 grams.

Symptoms as described under the "general symptoms" were observed. The eyes were severely affected, and degeneration thereof was obvious, as well as turbidity of the cornea and keratinization of the eyelids. There was a catarrhal discharge of the nasal chambers, and laboured breathing, due to sensitivity towards heat. This rat also showed deafness and took no notice of noises.

Curative treatment resulted in the rat gaining its normal vigour and health. The eyes very quickly (in a few days) became bright and open. The appetite was restored. In feeding vitaminized rat stock ration the animal gained 17 grams body weight in 12 days.

The rats in all cases (one in extremis died overnight) responded to treatment with vitamin A in the form of fish liver oil diluted with olive oil to an appropriate strength. At first the oil was given orally, a few drops at

a time. Later the feed was vitaminized with a few drops of oil, and as the appetite became normal again the balanced rat stock ration of this Institute was given.

#### SUMMARY.

1. Rats of 5-6 weeks of age with a vitamin A-reserve of about 1,200 I.U., when fed a vitamin A deficient ration depleted their reserves in the livers in 24 weeks time.

2. Characteristic symptoms were observed in the rats of the same ages after the 26th week when fed the same vitamin A-deficient ration; these are described in detail.

The rats when given curative treatment by dosing vitamin A responded very readily and regained normal vitality and appetite. Body weight increases were recorded.

#### ACKNOWLEDGMENTS.

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GRAPH I.

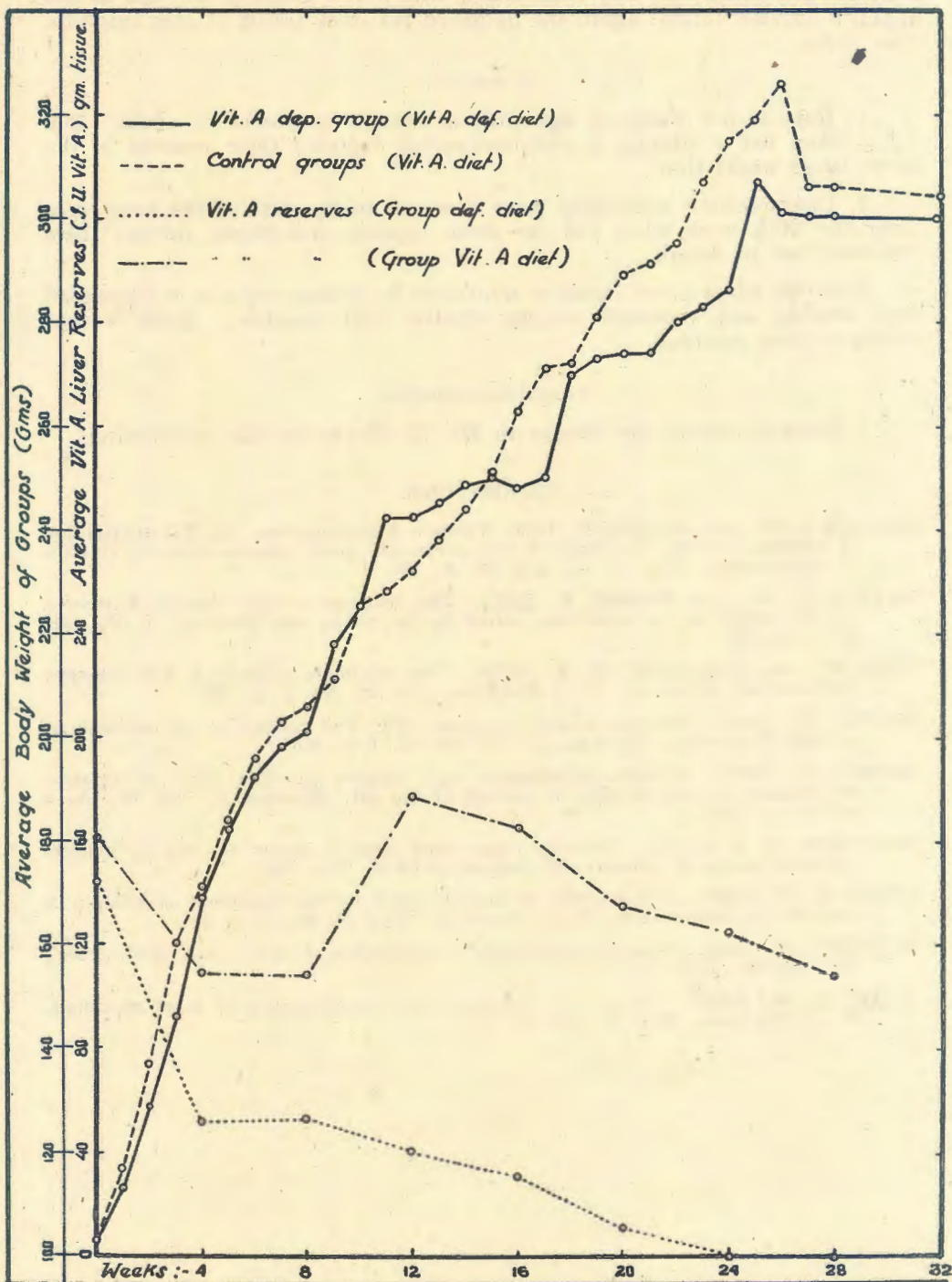




Fig. 1.

Fig. 1.—Control Group. Weight 296 gms. Age 8½ months.



Fig. 2.

Fig. 2.—Rat No. 2. Vitamin A deficient Group. Weight 180 gms. Age 8½ months.

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Fig. 4.



Fig. 3.

Fig. 3.—Rat No. 3. Vitamin A deficient Group. Weight 202 gms. Age 8½ months.

Fig. 4.—Rat No. 4. Vitamin A deficient Group. Weight 198 gms. Age 8½ months.