The Biologic Activity of Vitamin A Acid

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VITAMIN A ACID was first tested biologically by Arens and Van Dorp,[1] who isolated crystalline vitamin A acid as an intermediate in their synthesis of vitamin A. On feeding vitamin A acid to deficient rats, they found that the animals promptly began to grow again and were relieved of their deficiency symptoms. Arens and Van Dorp first assumed that this activity was the result of reduction of the acid in the body to vitamin A alcohol. To check this assumption, they gave massive doses of vitamin A acid to depleted animals and extracted their tissues for vitamin A. Regardless of the amount of acid administered, however, no vitamin A alcohol could be detected in the livers of these animals.[2] Consequently, they suggested that perhaps the acid itself is biologically active and is not reduced *in vivo*.

Shortly thereafter, Sharman[3] confirmed that vitamin A acid has intense growth-promoting power. Sharman likewise was unable to detect any vitamin A alcohol in the tissues of acid-dosed rats, and reported also that he was unable to detect any vitamin A acid in the tissues. Sharman agreed with Arens and Van Dorp that vitamin A acid probably exerts its activity without being reduced to either vitamin A alcohol or aldehyde.

Moore,[4] commenting on these findings several years later, pointed out that if vitamin A acid exerts its activity without being reduced *in vivo*, then it is unlikely that the acid can replace vitamin A in the visual cycle, which demands the alcohol or aldehyde as precursor of the visual pigments.[5]

We have recently shown that Moore's hypothesis is correct.[6] Young weanling rats raised on a vitamin A deficient diet supplemented with vitamin A acid become highly nightblind, and eventually completely blind, though continuing to grow normally and to remain otherwise in excellent condition.

Figure 1 summarizes the results of one such experiment. Two weanling animals were raised on the vitamin A deficient diet, but one animal was supplemented with vitamin A acid from the start of the experiment. Both animals grew at about the same rate for five to six weeks. Then the unsupplemented animal stopped growing, rapidly lost weight, and died on the fifty-seventh day of the experiment. The other animal continued to grow and appeared to remain in excellent condition, as the photograph taken on the one-hundred-fifty-seventh day of the diet is intended to show.

On the same day the rat's electroretinogram (ERG) was recorded. This is shown at the right of the figure, compared with that of a normal animal measured at the same time. The vitamin A acid animal, though normal in weight and appearance, was highly nightblind. Its visual threshold (the luminance of a 1/50 second flash needed to excite a just measurable ERG) was raised about 3.25 log units (about 1,800 times) above normal. Rhodopsin extracted from the retinas of animals in this condition is present in only 1 to 3 per cent of normal amounts.

Animals raised on a vitamin A deficient diet supplemented with vitamin A acid grow as fast and as well as animals on the deficient diet supplemented with vitamin A alcohol. We have compared the growth and appearance of groups of vitamin A acid- and alcohol-supplemented animals for periods of five to six months, and find them similar in all respects.[6] The gross appearance of the internal organs on autopsy, and the histologic appearance of the tracheal epithelium is the same in both groups.

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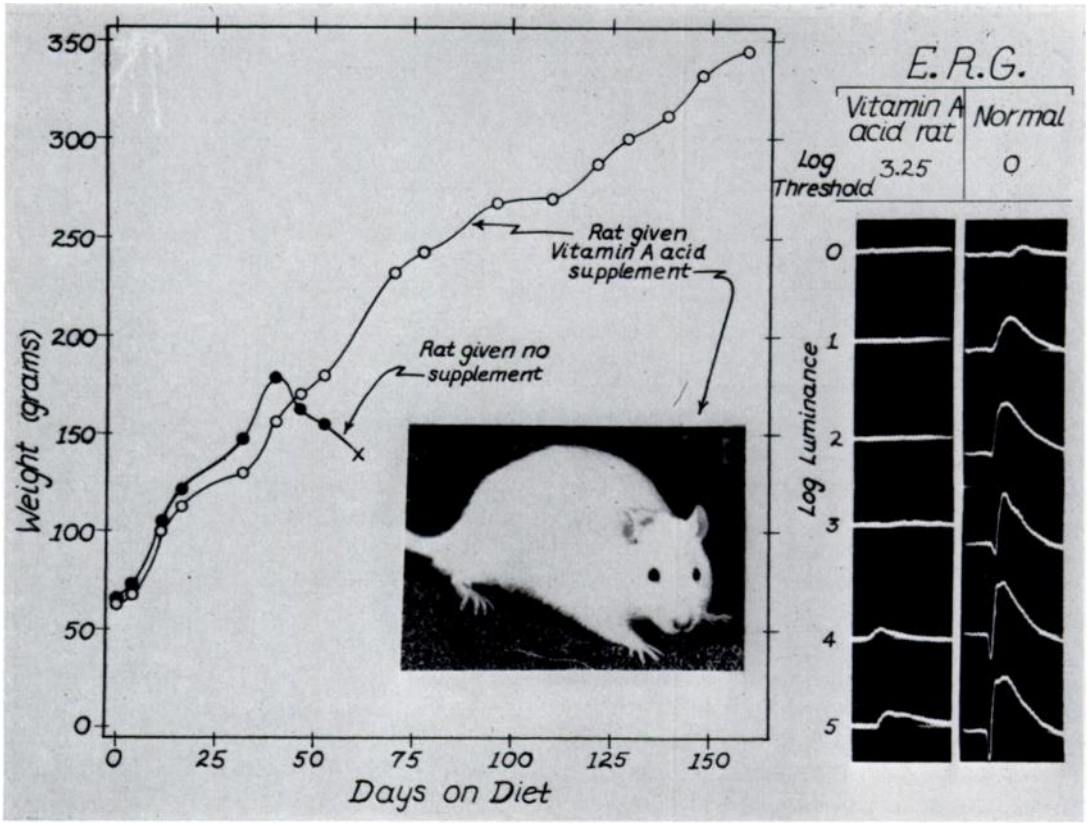

FIG. 1. Activity of vitamin A acid. Weanling rats were placed on a vitamin A-deficient diet, but one was supplemented with vitamin A acid. The rat given no supplement died after fifty-seven days on the diet; the animal receiving vitamin A acid continued to grow and remained in excellent condition for the duration of the experiment, a little over five months. The picture of this animal was taken at the end of the experiment, as were the electroretinograms shown at the right, compared with those of a normal animal. They show this rat to be highly nightblind: its visual threshold had risen 3.25 log units (1,800 times) above normal, and only just detectable ERG's could be evoked even at the highest luminances.

At the time of writing this report, we have observed one rat on this regimen for eighteen months, and it still appears perfectly healthy.

For our experiments, the supplements of vitamin A acid are dissolved in vegetable oil and given orally three times a week by means of a syringe fitted with a blunted needle. In most experiments, we feed at a dosage level of 50 μg. of vitamin A acid per day. This high level was chosen with the thought that if the acid possesses the lowest activity yet reported, about 10 per cent of vitamin A, we would still provide the equivalent of at least twice the minimal daily demand for vitamin A.

In recent experiments we* have raised groups of animals on vitamin A-deficient diets supplemented with 0.5, 5, 50, and 500 μg. of vitamin A acid per day. The supplements were first administered a few days after the animals had begun to level off in weight. At a dosage level of 0.5 μg. per day, little if any vitamin A activity is noted: the animals have all died within a few days after control animals fed no supplements or fed vegetable oil alone. The animals fed supplements of vitamin A acid at dosage levels of 5 or 50 μg. per day, however, quickly resume growth, continue to grow at the normal rate, and assume a normal appearance.

Rats fed at the dosage level of 500 μg. per day also begin growing again immediately, but continue to grow for only one to three weeks. They then level off in weight, begin to show signs of hypervitaminosis A (such as

* I should like to thank Mr. David Jewett for assisting with these experiments.

bleeding about the nose and eyes, loss of hair, etc.), and after five to six weeks all have died. This result confirms the report of Thompson and Pitt that high doses of vitamin A acid readily produce signs of hypervitaminosis A.[7] Thompson and Pitt have also shown that vitamin A acid causes symptoms of hypervitaminosis A at considerably lower dosage levels than does vitamin A itself.

On extracting the tissues of animals fed various levels of vitamin A acid, we have confirmed Arens and Van Dorp, and Sharman, in failing to detect any vitamin A or vitamin A acid in liver, blood, or kidney.[6] Redfearn[8] has also recently made similar observations, but finds that after large doses of the methyl ester of vitamin A acid, one can detect small amounts of this substance in the body fat, though none in the liver or kidney.

One consequence of the failure of animals to store vitamin A acid is that the animals can not long withstand interruption of the supplements. Within a week the animals begin to level in weight and to show signs of the deficiency. If supplements are not resumed, the animals lose weight rapidly and die within three to four weeks.[6] Redfearn has confirmed this observation, but reports that rats fed the vitamin A acid methyl ester tolerate the interruption of this supplement for longer periods of time.[8]

Supplements of vitamin A acid also do not conserve stores of vitamin A alcohol already present in the liver. We had noted that animals supplemented with vitamin A acid from a few days after weaning become nightblind as rapidly as animals given no supplements.[6] During the first few weeks on a deficient diet, animals deplete their livers of vitamin A; and it appeared from the preceding observation that this depletion might occur at the same rate whether vitamin A acid was administered or not. We confirmed this suspicion by measuring directly the disappearance of vitamin A from the liver. It turned out that the vitamin A content of the liver declines at the same rate in unsupplemented and vitamin A acid-supplemented groups of animals.[6]

As already said, the rise of visual threshold, nightblindness, occurs as promptly and develops at the same rate in animals supplemented with vitamin A acid as in animals receiving no supplements. After sixty days or so, however, animals receiving no supplements have all died, while animals receiving vitamin A acid remain well but continue to become more nightblind. Eventually (in eight to ten months), these animals become completely blind. At this time we no longer can record any electrical responses from the eyes, even with stimuli 7 log units (about 10 million times) above the threshold level. We can also no longer detect any rhodopsin in these retinas.[6]

The structure of the visual cells in the retinas of these animals also gradually degenerates as the night blindness progresses.[9] The degeneration begins in the outer segments of the rods (after two months or so) but eventually includes the rest of the visual cell, so that, after ten months, the visual cells have completely disappeared except for a few residual nuclei, pressed between the bipolar cells of the retina and the pigment epithelium. The rest of the retina (ganglion and bipolar cells, and pigment epithelium) remain normal in appearance; only the visual cells are affected in this condition.

If vitamin A is given to animals supplemented with vitamin A acid, visual recovery occurs in varying degree, depending on the extent of retinal degeneration. Visual cells which have not deteriorated too greatly can regenerate outer segments, but entire visual cells which have been lost are not replaced.[9]

It appears from these experiments that the only role for which specifically vitamin A is required in the rat is as precursor of the visual pigments. For all other, systemic functions of the vitamin, vitamin A acid seems to serve equally well. It has been suggested that vitamin A acid may either be the active principle for the tissue functions of vitamin A or an intermediate between vitamin A and the active principle.[4,6] The metabolism of vitamin A can be expressed in such a diagram as shown in Figure 2.

Vitamin A is stored in the tissues as vitamin A ester and is transported as the free alcohol. It may be reversibly oxidized to vitamin A aldehyde (retinene) to form the visual pigments. It appears to be irreversibly oxidized to vitamin A acid, which may either serve as

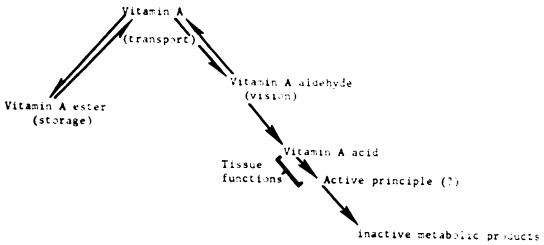

FIG. 2. General metabolism of vitamin A.

the active principle or may be converted to the active principle.

The failure to detect vitamin A acid in the tissues of rats dosed with this substance may favor the latter hypothesis and has prompted us to test possible oxidation products of vitamin A acid for growth-promoting potency. We* have now tested the C_{19} acid, the C_{18} ketone, the C_{17} acid and the C_{14} aldehyde, but have not found any of these compounds to display vitamin A activity.

SUMMARY

The literature concerning the activity and metabolism of vitamin A acid has been reviewed. Recent experiments testing the potency of vitamin A acid at various dosage levels are reported, along with experiments testing the vitamin A activity of several possible oxidation products of vitamin A acid.

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* I should like to thank Mr. David Jewett for assisting with these experiments.

DISCUSSION

DR. G. J. WRIGHT (*Urbana, Illinois*): When the work of Drs. Dowling and Wald on vitamin A acid became known, we naturally were interested to see if we could detect the presence of this acid in any of our systems. Using the technic of carrier crystallization, we attempted to detect the formation of radioactive vitamin A acid from C^{14}-labeled vitamin A acetate in normal pig adrenal homogenates and in tissues of rats which had been injected with the C^{14}-labeled material. The results of these experiments are shown in Tables I and II. As can be seen from these data, no vitamin A acid could be detected. It seems reasonable to assume that if vitamin A acid were the "active" form of the vitamin its presence could be detected by these means. These experiments, of course, do not preclude the possibility that vitamin A acid is an intermediate with an extremely rapid turnover rate.

TABLE I

Nonformation of Vitamin A Acid by Adrenal Homogenates*

Crystallization	Recovered Carrier (mg.)	Specific Activity (c.p.m./mg.)	Total Activity (c.p.m.)
...	5	...	2,560
1	3.9	104	406
2	2.2	220	44
3	1.2	0	...

* Each incubation included 3 ml. of homogenate, adenosine triphosphate, 2×10^{-3} mM., TPNH, 1.4×10^{-3} mM., vitamin A acid, 15 μg., 0.05 μc. vitamin A-1′, 9-C^{14} acetate (7 μg).

TABLE II

Vitamin A Acid Carrier Crystallization with Water-soluble "Metabolite" of C^{14}-Vitamin A Acetate from Rat Liver

Crystallization	Recovered Carrier (mg.)	Specific Activity (c.p.m./mg.)	Total Activity (c.p.m.)
...	43.4	...	13,300
1	40.6	76	3,085
2	23.5	14	329
3	12.3	0	...