

PATHOLOGIC CHANGES OCCURRING IN WHITE  
RATS RAISED ON DIETS DEFICIENT IN  
VITAMIN A\*

IRA A. MANVILLE, M.D.

PORTLAND, ORE.

It now seems well established that there are two distinct factors concerned in the Fat-Soluble A vitamin. One is antirachitic. The other is antixerophthalmic and growth promoting, and retains the appellation vitamin A.

The bony lesions following a dietary regimen in which the antirachitic factor has been lacking have been described.<sup>1</sup> Among other lesions resulting from this deficiency, Kauffman, Creekmur and Schultz<sup>2</sup> have described changes in the middle ear resulting in varying degrees of deafness. Grieves<sup>3</sup> also claims that dental caries is another manifestation of the upset in calcium metabolism.<sup>4</sup> There has also been mentioned in connection with rickets of a certain type an upset in nervous equilibrium due to an improper calcium balance,<sup>5</sup> while Reynolds and Macomber<sup>6</sup> assign to the same cause some cases of sterility.

The absence of vitamin A from an otherwise adequate diet will cause a cessation of growth soon followed by the characteristic eye lesions so well described by Mori.<sup>6</sup> He also showed that the salivary glands undergo a cessation of secretory activity analogous to that undergone by those of the orbit. Daniels, Armstrong and Hutton<sup>7</sup> have recently emphasized the fact that the lack of vitamin A is the specific

\* From the department of physiology, University of Oregon Medical School.

1. Shipley, P. G.; Park, E. A.; McCollum, E. V., and Simmons, Nina: Is There More Than One Kind of Rickets? *Am. J. Dis. Child.* **23**:91-106 (Feb.) 1922.

2. Kauffman, A. B.; Creekmur, Francis; and Schultz, O. T.: Changes in the Temporal Bone in Experimental Rickets, *J. A. M. A.* **80**:681-685 (March 10) 1923.

3. Grieves, C. J.: The Effect of Defective Diets on Teeth, *J. A. M. A.* **79**:1567-1573 (Nov. 4) 1922.

4. We have noted in our own work a greatly increased fragility of the teeth of deficient rats, and, in some instances, we have found a stub where a brittle tooth had snapped off.

5. Reynolds, Edward; and Macomber, Donald: Defective Diet as a Cause of Sterility, *J. A. M. A.* **77**:169-175 (July 16) 1921.

6. Mori, S.: Changes in Para-Ocular Glands Which Follow Administration of Diets Low in Fat-Soluble A; Effect of Same Diets on Salivary Glands and Mucosa of Larynx and Trachea, *Bull. Johns Hopkins Hosp.* **33**:357-359 (Oct.) 1922.

7. Daniels, Amy L.; Armstrong, Margaret E., and Hutton, Mary K.: Nasal Sinusitis Produced by Diets Deficient in Fat-Soluble A Vitamin, *J. A. M. A.* **81**:828-829 (Sept. 8) 1923.

factor that permits another disorder to develop which they have termed paranasal and mastoid sinusitis. Because of this deficiency, bacterial invasion of the mucous membranes of the ear and nasal cavities is made possible.

The results of experiments with vitamin A deficient diets seem to justify two general statements: first, that cellular activity is minimized, and second, that out of this reduction in activity comes an increased susceptibility to infections hastening the termination of the experiment. A finer analysis of the first of the preceding statements will show that the results of a lessened cellular activity will depend on the type of cell affected. If there is a curtailment of the activity of somatic cells, the first result will be failure to produce new cells—stoppage of growth; the second, inability to replace cells already existing—loss of maintenance, and the third, actual regression. If there is a diminution in the activity of glandular cells, the results will vary with the type of gland affected and, in general, there will be a lessened resistance to infection, malnutrition and sterility.

Vitamin A is a substance necessary for the normal functioning of the body tissues. It is not clear just how it meets this necessity. It may either be one of the essential ingredients needed in cell structure or it may act as a hormone making available some necessary component in cell formation. In either case, it is absolutely essential for the cell multiplication necessary for growth and maintenance.

In general, growth is proportional to the supply of vitamin A. Normally, a rat matures at three months of age. It also obtains about three fourths of its body weight in that time. At the very time in a rat's life when the bulk of the vitamin A supply is no longer needed for growth, the reproductive organs begin to function. Most of the vitamin A intake during the first three months of a rat's life is used for the formation of new cells throughout the body. When the animal has practically attained its growth and the majority of the new somatic cells needed are those required for replacement, the vitamin A supply is utilized in new quarters, in the glandular elements, where cell increase suddenly takes a tremendous impetus, which it maintains throughout the vigorous span of a rat's life. The rat is now mature; the sexual function is established, and the nervous, respiratory and digestive systems have become stabilized by proper and sufficient glandular activity.

The purpose of this paper will be to offer observations in support of the preceding statements, and to show that a vitamin A deficiency causes such a reduction in cellular activity as to amount to a pluriglandular deficiency, and that this in turn brings about secondary changes directly leading to death.

The technic of the feeding experiments conforms as closely as possible to the rules governing such experimentation. A description of methods has appeared so many times in the literature that, aside from saying no departures were made from the best approved methods, nothing further will be added.

Our work confirms in many respects the findings mentioned above, and brings out additional pathology of this deficiency. For some time, we have observed that our rats on a vitamin A low diet, when reaching a stationary nutritive regimen, could be made to do much better by the simple expedient of moistening their food. The food is ordinarily in a semidry, caked condition. For the first seven or eight weeks, they eat enough of the food in this condition to produce fair growth curves. Then ensues a period of variable length, from one to three weeks, when they either become fussy and scatter the food about the cage or act indifferently toward it. If sufficient moisture is now added to the food, this condition does not become so acute. As implied above, we are inclined to attribute this to the xerostomia and to consider it an early sign of the specific vitamin deficiency. This dry condition of the mouth is further demonstrated in the following manner: Several small cotton swabs of uniform size and firmness are made. They are slightly moistened in a solution of brom thymol blue, which will turn blue when in contact with the alkaline saliva. One swab is used for each rat. An equal number of normal rats are similarly treated for controls. The moistened applicator is placed in the rat's mouth and thoroughly swabbed about. In the normal animals, the cotton immediately takes on a very dense blue color, while with the test animals the color appears fainter in hue and occurs in spots and patches, indicating a lessened amount of salivary secretion.

With the procedure just described, a fairly accurate estimate may be obtained of the hydrogen ion concentration of the saliva, of the secretions of the vagina and the rectum, of the peritoneal fluid and of the blood serum. In such instances, the swabs were matched for color to a series of swabs made from buffer solutions of known hydrogen ion concentration. Thus it was that we found that the reaction in the mouth of a rat suffering from xerophthalmia and xerostomia had changed from the normal alkaline condition of  $p_{H}$  7.4 to an acid one of  $p_{H}$  6.6. This was due to a decrease in the alkaline saliva, increased stasis of intra-oral material and bacterial activity.

This condition of dryness does not exist in the mouth alone. Mori<sup>6</sup> found it in the eyes. We have found that it involved the upper part at least of the trachea, for such rats, when given painful stimuli, are unable to squeal as do normal rats when similarly stimulated, but give forth, instead, a dry, husky cry or none at all, depending on the degree of

involvement. This attempt at a cry is accompanied by the usual facial grimaces, and is undoubtedly an attempt on the part of the rat to give voice to his discomfort. This dysarthria is additional evidence of the general xerosis due to glandular insufficiency, and is analogous to a similar condition, described by Mori,<sup>9</sup> occurring in school children suffering with xerophthalmia.

This lack of glandular activity does not seem to be limited to the para-ocular, paranasal and para-oral glands. The dryness of the feces and of swabs applied to the rectum, large bowel and vagina seems to indicate that here, too, is a lessening in the secretions. There are still other evidences of vitamin A deficiency that can very well be associated with the lack of glandular activity noted in the foregoing. These are the lack of gonadal activity as manifested by variations in reproductive ability, marked underdevelopment of the seminal vesicles, lessening of the mammary secretion, due both to incomplete development and to decreased function, and a curtailment in the secretory activity of the oil glands of the skin, resulting in a dry, bristling hair, which falls out much more readily than that of normal animals. More could be said concerning the changes occurring in the reproductive system, but this material is being reserved for a future report.

The marked decrease in cellular activity noted above should be considered the primary pathologic change resulting from vitamin A deficiency. The decrease in secretions promotes desquamative changes; destroys the action of cilia; lowers bactericidal ability, and, in many other ways, provides portals for entry of numerous organisms that soon produce pyogenic lesions with their associated complications. These should be considered as secondary pathologic changes resulting from a lack or shortage in vitamin A.

Our necropsy records of such deficient rats show that a large majority of them had pyogenic infections. In many instances, we found the posterior third of the tongue the seat of abscesses ranging in severity from a single small centrally placed abscess to an involvement in which there was almost total destruction of that portion of the tongue. The organisms causing these central abscesses seemed to have gained entrance either through the foramen cecum or that tissue representing its vestiges. Other abscesses, more laterally placed and generally much more extensive and destructive, had their origin from material escaping from the ruptured salivary ducts leading from the submaxillary and sublingual glands. In all cases of lingual abscesses, the sublingual and submaxillary glands were found infected, their ducts greatly distended with purulent material and the region of their opening beneath the tongue always marked by cystic dilatation. Other abscesses have been found on the borders of the lips. These oral abscesses must be of prime importance

as reasons for the rats' refusal to eat, and undoubtedly share in the explanation of their nutritive decline.<sup>8</sup>

In most cases, mucopurulent material has been found in the nasal sinuses and middle ear due to direct extension from the nasal tracts and eustachian ducts. Large purulent plugs have been found in the nasopharynx which had their origin as drainage from the higher respiratory areas. These plugs frequently acted as a serious menace to free respiration. In these particular instances, the rats would have paroxysmal spells of sneezing, which would produce marked cyanosis and pronounced exhaustion. It is likely that during these paroxysms particles became dislodged and were aspirated further into the respiratory tract, producing numerous purulent deposits in the lungs. Necropsies on such animals seemed to indicate such a course. Thus, the infection was spread to the pulmonary tissues, and extensive empyemic areas developed. We have seen numerous cases in which the lung tissue was almost entirely replaced by immense accumulations of pus. These animals did not seem to die so much from the infection itself as from the gradual obliteration of the respiratory tissue and the resultant asphyxia. Respirations would become more dyspneic, and oxygenation so poorly accomplished that the rat would have to minimize all physical effort. Even then he would gradually become more cyanosed; lose

---

8. I was an interested witness at a necropsy recently held at the Multnomah County Hospital. The body examined was that of a Chinese, aged 55. He was 5 feet 9 inches (175.2 cm.) in height, and weighed 100 pounds (45.4 kg.). His body was very emaciated. No clinical diagnosis had been made, as he persistently refused to answer all questions, stating over and over again that he was all right. Physical examination was negative, except for small enlargements on both sides of the throat in the region of the submaxillary glands. The teeth were extremely bad. The urine was negative, except for a trace of albumin and a few casts. The history, so far as obtained, was also negative. He was in the hospital only a few days before he died, and during that time steadily refused all food, drink and medication. Toward the last, he even refused to have his temperature taken by mouth. He talked very little, and then only by urging. The temperature averaged 97 F., running at times as low as 94 and as high as 98.2. The pulse was 80, and the respirations 20. The only complaint was a "burning in the throat." He rapidly grew weak, and toward the last was occasionally irrational. Outside the usual senile changes and evidences of arteriosclerosis, the necropsy findings were negative, except for these very significant points: A large abscess beneath the tongue was found at the point of exit of the submaxillary ducts; the ducts themselves were distended with pus and the glands were greatly enlarged and engorged with purulent material; the esophagus presented numerous submucous abscesses, and the posterior portions of the lungs were congested. Although the cause of death was given as hypostatic pneumonia, it is evident that this is only a partial truth and does not explain the primary condition leading to this result. It is difficult to understand how a human being living in the midst of our modern life could die from a deficiency such as described above, yet it must be considered a possibility. Especially is this true when one considers how near to the border of adequacy are many of the menus of an average person's diet.

steadily in body heat, and, finally, die of exhaustion and malnutrition and the associated low grade intoxication.

By direct extension from the nasopharynx, the infection spread to and involved the middle ear, and, in some cases, became so severe in this location that the vestibular apparatus also became involved, causing the animal to show circus movements. Frequently, the stomach is found containing purulent material which has been swallowed from the nasopharynx. Small abscesses have been found on the borders of the eyelids; others at the bases of the nails, and, in one case, a large pus pocket was found embedded in the wall of the vas.

In regard to the multiple purulent onychia noted above, it should be mentioned that Haden and Jordan<sup>9</sup> have reported experimental work in which they claim that multiple onychia can be produced as a result of a blood borne infection having as a source septic foci in the teeth. Grieves<sup>3</sup> has found that such infective foci exist as a result of these defective diets. It would therefore seem probable that this case of multiple purulent onychia is an exceptional graphic demonstration of the generalized infection that affects many of these deficient rats.

Mention has been made elsewhere in the literature of the fact that normal rats, under laboratory conditions, occasionally develop a peculiar infection of the lungs that causes death. This condition is similar to that described above but is in less aggravated form and only occurs: (a) in old rats; (b) in those which have been subjected to too heavy a reproductive strain and, as a result, have their resistance lowered to a critical point, and (c) in those whose vitality was never on a par with that of their mates. Any change from the normal habitus of an animal will make it more susceptible to the inroads of disease, and, for that reason, only the most robust and vigorous should be selected for experimental purposes. It must not be forgotten that the same tendencies are inherent in vigorous animals, and that it takes only some additional load, such as a defective diet, to make the same diseases appear in them.

It will thus be seen how the decrease in the secretory elements of the eyes and mouth lowers the resistance to invading organisms which soon dominate the situation with serious results. Histologic sections of the trachea reveal a desquamated and eroded condition. In the lungs, the alveoli can be seen breaking down to form larger cavities. Wherever the lining of the alveoli is eroded or broken through, numerous organisms can be seen invading the surrounding tissue. This infection cannot be of pronounced virulence, for occasionally normal animals develop the snuffles and recover with no further extension of the infection. The

---

9. Haden, R. L., and Jordan, W. H.: Multiple Onychia as a Manifestation of Focal Infection, Arch. Dermat. & Syph. 8:31-36 (July) 1923.

test rats become affected in the same degree as their diet is deficient. If the diet is only moderately deficient or if the animal is of greater vigor, the infection is overcome but recurs again and again with increasing severity as the deficiency becomes more and more pronounced.

The lung condition above described is decidedly at variance with another pulmonary involvement which also occurs but in which the pyogenic agency does not play so prominent a part. Rats dying of the latter condition do not run so long a course. The process is more fulminating and is not always associated with a metabolic decline; that is, their growth curves may still be on the ascent. They will, nevertheless, generally show slight pyogenic involvement at the base of the tongue or in the ears. The nose, however, instead of containing mucopurulent material, has a serosanguineous exudate which discharges quite freely from the nares. The tracheal mucosa is inflamed and covered with a sanguineous exudate. The lungs are extremely congested. This is evidently a bronchogenic pneumonia of a sufficiently virulent type to overwhelm the animal before much loss in body weight has occurred.

The fatalities resulting from the infections described in the foregoing are not due, according to Werkman,<sup>10</sup> to any reduction in the cataphylactic activity of the animal. Findlay<sup>11</sup> showed that in vitamin B deficient pigeons, the resultant loss in body heat was the decisive factor in the development of fatal pneumonias. In our animals, loss of heat could not have been a factor since their temperatures did not decline from the normal 101 to 102 F. for many days after the infection was obviously present. In fact, a drop in temperature was almost always associated with a slowing of the respiratory rate and the two together considered as early signs of death. We have frequently observed rats with a body temperature so low as not to be obtainable with an ordinary clinical thermometer and with respiratory rates of 16 to 20 a minute. Any influence of a possible resultant thrombopenia on susceptibility to infection cannot be claimed as the evidence is too conflicting.<sup>12</sup>

#### COMMENT AND SUMMARY

In general, the results of test feeding depend to a great extent on the time in a rat's life when the experimental dietary is commenced. We

10. Werkman, C. H.: Immunologic Significance of Vitamins; Influence of Lack of Vitamins on Production of Specific Agglutinins, Precipitins, Hemolysins and Bacteriolysins in Rat, Rabbit and Pigeon, *J. Infect. Dis.* **32**:247-254 (April) 1923.

11. Findlay, G. M.: B Vitamin and Pneumococcal Infection, *Lancet* **1**:714-715 (April 8) 1922.

12. Cramer, W.; Drew, A. H., and Mottram, J. C.: Behavior of Platelets in Vitamin A Deficiency and Technique of Counting Them, *Brit. J. Exper. Path.* **4**:37-44 (April) 1923. Bedson, S. P., and Zilva, S. S.: Influence of Vitamin A on Blood Platelets of Rat, *Brit. J. Exper. Path.* **4**:5-12 (Feb.) 1923.

wish to emphasize the fact that on this point depends whether or not the results noted are due to functional or to organic changes. If a rat is used for experimental feeding tests after it has attained its growth, the results will be more or less due to functional disturbances, while those obtained with rats that are fed test diets during the growing stage will be due to organic changes. Especially is this true of vitamin A underfeeding. A rat raised on a diet lacking only in vitamin A has a pluriglandular deficiency in substance as well as in secretion such that normality cannot be established by any increase in the vitamin A fraction. The best that could be expected on an optimal diet would be a maximum efficiency for what tissue it possesses. On the other hand, adult rats whose tissues have matured but are in a nonfunctioning condition because of the lack of these essential elements, can be restored to their original activity when the missing factors are supplied.

Stress is laid on the point that not only must a diet be adequate in all respects but that it must also be presented to the body at a time when its life's forces can utilize it in the formation as well as the operation of its cellular units.

The pathologic condition arising from vitamin A deficient diets has been described under two heads, primary and secondary. The primary change occurring is a generalized decrease in glandular activity resulting in xerosis of the eye, mouth, larynx and skin, and in malnutrition and sterility. This makes possible the secondary changes that occur, namely, those produced in the eye, nose, mouth and lungs by bacterial invasion.

*Reprinted from the Archives of Internal Medicine*  
*May, 1925, Vol. 35, pp. 549-556*

COPYRIGHT, 1925  
AMERICAN MEDICAL ASSOCIATION  
FIVE HUNDRED AND THIRTY-FIVE NORTH DEARBORN STREET  
CHICAGO