VITAMIN A DEFICIENCY AND METAPLASIA.

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INTRODUCTION.

Mori (1) was the first to draw attention to the change which occurs in the lining epithelium of the larynx, trachea, and ducts of the Meibomian, submaxillary, sublingual, and parotid glands of rats that have been fed a diet deficient in the fat-soluble vitamins. He referred to the change as keratinization, and, as in the case of xerophthalmia (2), attributed it to the drying of the epithelium (xerosis) due to the hypofunction of the glands whose secretion keeps these epithelial surfaces moist. The impaired activity of these glands he considered to be due to the pathological changes (atrophy and degeneration) induced in them by the vitamin deficiency.

Wolbach and Howe (3, 4) recently confirmed the findings of Mori (1), examined many more organs and tissues than did Mori, and found the changes in some of them, as well as in some (pancreatic duct and renal pelvis) in which Mori had not found any. Wolbach and Howe referred to the change as a metaplasia of the cylindrical or cuboidal or transitional type of epithelium to the squamous keratinizing type, and attributed to the fat-soluble vitamin deficiency the direct specific cause of the metaplastic changes. They also found the other morphological changes described by Mori in paraocular, submaxillary, and other serous and mucous glands, but did not consider that the lack of the secretions of these glands induced the metaplasia.

The diets employed by Mori (1) and by Wolbach and Howe (4), although referred to by them as deficient in fat-soluble A, were actually deficient in vitamin A (antiophthalmic, growth-promoting), vitamin C (antiscorbutic), and vitamin D (antirachitic), but this fact was recognized by the authors.

Since it has been shown that xerophthalmia will develop as a result of a dietary deficiency of vitamin A alone, and since the diets employed by Mori and by Wolbach and Howe were deficient in several vitamins, it seemed of interest to us to determine whether a single vitamin deficiency (vitamin A) could also induce the metaplasia and other changes in glandular organs, that have been described by these investigators.

EXPERIMENTS.

The Animals.

A total of 63 piebald black and white rats bred in this institution were used in this investigation. The sex, number of animals in every group, initial, maximal, and final weights, and length of time on the special diets are given in Tables I to III. Most of the rats were from 28 to 35 days old at the beginning of the experimental period. The animals were kept in individual wire mesh cages in a well ventilated room protected from direct sunlight. Representatives from every litter were put into three groups. Group I received a diet deficient in vitamins A and D (Diet −A−D), but complete in all other respects; Group II received a diet deficient in vitamin A alone (Diet −A); and Group III received a diet complete in every respect (Diet +C. L. O.).

The Diets.

Group I.—(Diet −A−D.) This diet is deficient only in vitamins A and D, and consists of the following:

Inactivated* technical casein (Merck)	20 gm.
Corn-starch	50 gm.
Cottonseed oil	15 gm.
Salt mixture, McCollum No. 185 (6)	5 gm.
Vegex (Marmite) (for vitamin B)	5 gm.
Decitrated lemon juice (for vitamin C)	5 cc.
Distilled water	50 cc.

* By heating and aeration (Goldblatt and Moritz (5)).

Group II.—(Diet −A.) This diet is deficient only in vitamin A. The ingredients of the diet are exactly the same as those of Group I, but in order to supply the antirachitic factor, the cottonseed oil was irradiated by a mercury vapor quartz lamp in a manner described before (Goldblatt and Moritz (7)).

Group III—(Diet +C. L. O.) The diet is the same as that of Group I, but in order to supply the missing vitamins A and D, and to make the diet complete in every respect, 7 drops (about 150 mg.) of cod liver oil (Harris) were administered daily by mouth to every rat in this group.

The rats of Groups I and II ate from 10 to 20 gm. daily in the beginning, and from 7 to 15 gm. later, while those in Group III ate from 15 to 25 gm. daily throughout the experimental period. The animals were sacrificed after they had been on these diets for varying periods. (See Tables I to III.)

Autopsy Findings.

The only gross morbid changes seen were occasional lobular pneumonia in the three groups, abscesses in the base of the tongue in practically every rat of Groups I and II in which this organ was examined, xerophthalmia in some of the rats of Groups I and II, an occasional abscess in the submaxillary gland in Groups I and II, and in a few instances, in Groups I and II, dilated ureters or dilated external bile ducts.

The tongue abscesses are of special interest. They have been described by Sherman and Munsell (8) who found them in 76 per cent of their rats on a diet deficient in the fat-soluble organic factor. Wolbach and Howe do not speak of abscess, but of cysts, and they say that: "Infection complicates the picture as it causes complete destruction of the epithelium lining cysts and repair by granulation tissue accompanied by foreign body reactions to the retained keratinized cells." The abscesses are probably painful and, more than the loss of sense of smell, mentioned by Wolbach and Howe, may explain, at least in part, the suddenness with which many of the rats cease to eat and in consequence lose much weight. Smears were made of the pus from these abscesses; organisms were always found, and practically always they were a mixture of Gram-positive diplococci and Gram-negative bacilli, some free, others inside pus cells. Occasionally, the only organisms were Gram-positive diplococci. Cultures in broth and on solid media showed the same organisms, but they have not yet been definitely identified. In no instance were abscesses of the tongue or submaxillary glands found in the rats of Group III (+ C. L. O.).

Histological Findings.

The tissues were either fixed in Zenker's fluid and stained with eosin and methylene blue, or fixed in formalin and stained with hematoxylin and eosin. In many instances half of an organ was treated by one

and the remainder by the other method. Both were very satisfactory for the recognition of metaplastic changes. Sections were made at various levels of an organ. The stomach was not investigated histologically. Keratinization of the cornea and conjunctiva alone was not considered a positive result.

In the case of about two-thirds of the rats of every group all the organs investigated by Wolbach and Howe were examined histologically, but in the remainder, only nasal passages, larynx, trachea, bronchi, lungs, and tongue were investigated microscopically. For our purpose this was deemed sufficient because, in those rats of Groups I and II in which all organs were examined, no metaplastic changes were seen in any other organ when there were none in the accessory salivary glands of the tongue and in the respiratory tract. Wolbach and Howe also state that the changes practically always appear first in the respiratory tract. Besides, in no rat of Group III (normal diet) was squamous keratinizing epithelium found in an abnormal situation (see Table III). Since we were using metaplasia as a morphological indicator, and since a positive finding in a single organ was adequate for our purpose, the results still further justify the procedure because the metaplastic changes were found in some part of the respiratory tract and in the accessory salivary glands of the base of the tongue in practically all the rats in Groups I and II (see Tables I and II). Similar changes were found in fewer instances in the ducts of paraocular, submaxillary, sublingual, and parotid glands, and in renal pelvis, ureter, and bladder. The manifestations were similar to those described by Mori and by Wolbach and Howe; they varied from small isolated nests to complete transformation of the epithelium in the sections examined, and appeared with or without accompanying inflammation. When inflammation was present, the metaplastic changes were usually more severe. Even in the case of the accessory salivary glands of the base of the tongue, often, in the one organ, there were large cysts lined completely, or in part, by squamous epithelium, filled with keratohyaline material, and without any signs of inflammation, while in another portion there was a large abscess lined partly, sometimes completely, by squamous keratinizing epithelium. It would appear therefore, that the inflammatory process occurs coincident with or subsequent to the metaplasia, and is not responsible for the initiation

of the changes. This is also the view of Mori and of Wolbach and Howe. Atrophy and degeneration of the epithelium of the testicular tubules and of the acini of the salivary and paraocular glands were a common finding in Groups I and II, but did not occur in Group III. This agrees with the findings of Mori and of Wolbach and Howe.

TABLE I.

Group I (Diet −A−D).

Number of rat	Sex	Days on diet	Weight			Metaplasia	Remarks
			Initial	Maximal	Final		
			gm.	*gm.*	*gm.*		
1	M.	137	52	174	149	Absent	Bronchopneumonia
2	M.	133	45	167	149	Present	Xerophthalmia
3	M.	140	45	162	142	Present	Xerophthalmia
4	M.	115	50	147	125	Present	Dilated ureters and bile ducts; xerophthalmia
5	M.	95	57	108	94	Absent	Xerophthalmia
6	M.	95	44	120	101	Present	Bronchopneumonia
7	M.	95	46	70	63	Present	Xerophthalmia; bronchopneumonia
8	M.	88	47	98	92	Present	—
9	M.	79	38	83	73	Present	—
10	M.	98	60	195	188	Present	Abscess of tongue
11	M.	70	55	136	110	Present	Abscess of tongue
12	M.	72	45	151	130	Present	Xerophthalmia; abscess of tongue
13	M.	77	40	142	115	Present	Abscess of tongue and submaxillary glands
14	M.	70	43	155	121	Present	Xerophthalmia; abscess of tongue
15	M.	83	55	110	95	Present	Abscess of tongue
16	F.	95	46	98	95	Present	—
17	F.	95	51	84	80	Present	—
18	F.	95	46	90	75	Present	Bronchopneumonia
19	F.	89	40	82	65	Present	—
20	F.	90	38	79	73	Present	—
21	F.	85	36	66	56	Present	Bronchopneumonia
22	F.	98	47	137	135	Absent	—
23	F.	98	51	147	145	Present	Xerophthalmia; abscess of tongue
24	F.	70	45	121	100	Present	Xerophthalmia; abscess of tongue
25	F.	70	35	113	100	Present	Abscess of tongue
26	F.	84	35	117	75	Present	Xerophthalmia

TABLE II.

Group II (Diet −A).

Number of rat	Sex	Days on diet	Weight			Metaplasia	Remarks
			Initial	Maximal	Final		
			gm.	*gm.*	*gm.*		
27	M.	101	44	166	120	Present	Xerophthalmia; abscess of submaxillary glands
28	M.	95	53	187	187	Present	—
29	M.	74	51	125	88	Present	Enteritis
30	M.	116	61	153	132	Present	—
31	M.	78	55	166	125	Present	Abscess of tongue
32	M.	91	35	181	150	Present	Abscess of tongue
33	M.	98	40	188	148	Present	Abscess of tongue
34	M.	63	42	135	113	Present	Abscess of tongue
35	M.	71	42	148	112	Present	Abscess of tongue
36	M.	56	55	120	102	Present	Xerophthalmia; abscess of tongue
37	F.	74	47	150	123	Present	Xerophthalmia; bronchopneumonia
38	F.	95	52	150	148	Absent	Xerophthalmia
39	F.	98	50	144	141	Present	Dilated ureters
40	F.	94	55	135	135	Present	Abscess of tongue
41	F.	78	48	129	97	Present	Abscess of tongue
42	F.	78	45	128	91	Present	Abscess of tongue
43	F.	91	33	128	97	Present	Abscess of tongue and submaxillary glands
44	F.	98	38	145	110	Present	Abscess of tongue; xerophthalmia

TABLE III.

Group III (Diet +C. L. O.).

Number of rat	Sex	Days on diet	Weight			Metaplasia	Remarks.
			Initial	Maximal	Final		
			gm.	*gm.*	*gm.*		
45	M.	144	52	240	208	Absent	Bronchopneumonia
46	M.	137	44	233	228	Absent	—
47	M.	95	48	228	170	Absent	—
48	M.	95	38	216	216	Absent	—
49	M.	95	40	253	253	Absent	—
50	M.	77	39	258	258	Absent	—
51	M.	77	37	241	241	Absent	—
52	M.	77	37	212	212	Absent	—
53	M.	56	50	168	168	Absent	—
54*	M.	77 + 11	45	250	146	Absent	—
55*	M.	56 + 16	43	222	124	Absent	—
56*	M.	77 + 9	45	240	147	Absent	—
57*	F.	56 + 16	40	150	94	Absent	—
58*	F.	77 + 11	45	280	166	Absent	—
59*	F.	77 + 9	40	150	96	Absent	—
60	F.	95	45	191	191	Absent	—
61	F.	95	42	174	174	Absent	—
62	F.	95	42	160	160	Absent	Bronchopneumonia
63	F.	77	35	170	170	Absent	—

* These rats were on diet (+C. L. O.) for the first period indicated in the column headed "Days on diet" and were deprived of all food except a mixture containing vitamins A, B, C, and D for the second period indicated in the same column.

TABLE IV.

	Number of rats in group	Number of rats showing epithelial metaplasia
Group I (Diet −A − D)	26	23
Group II (Diet −A)	18	17
Group III (Diet +C.L.O.)	19	0

Special Note.

Since, as is usual, many of the rats on −A and −A−D diets ate but little during the last week or two of their lives and often lost much weight, it was conceivable that the lack of food in itself might play some part in initiating the metaplasia. An experiment was therefore performed to test the effect of deprivation of food. Included in Group III (Diet + C. L. O.) are six rats (Nos. 54, 55, 56, 57, 58, and 59) which had been receiving Diet +C. L. O. for from 56 to 77 days and after that were deprived of food but were given an adequate amount of water and of vitamins A, B, C, and D in the form of a mixture of cod liver oil (about 150 mg.), Marmite (0.5 gm.), and decitrated lemon juice (0.5 cc.), which was administered daily by mouth. In no instance were metaplastic changes seen in any of the organs of these six rats, but atrophy of most tissues, even of the lining epithelium of the trachea and bronchi, was in most instances very great. Abscess of the tongue did not occur in these rats.

SUMMARY.

Of nineteen rats on a complete diet (Group III, Diet +C. L. O.), none showed foci of squamous keratinizing epithelium in abnormal situations.

Of twenty-six rats on a diet deficient in vitamins A and D (Group I, Diet −A−D), twenty-three showed metaplastic changes of varying degree in one or more organs; the metaplasia was of columnar, cuboidal, and transitional epithelium to the squamous keratinizing type.

Of eighteen rats on a diet deficient in vitamin A alone (Group II, Diet −A), seventeen showed epithelial metaplasia similar to that of Group I in one or more organs.

In Groups I (Diet −A−D) and II (Diet −A) the changes occurred in one or more of the following organs: trachea, large bronchi, small bronchi or bronchioles in lung, posterior nares, accessory salivary glands of base of tongue, paraocular, submaxillary, sublingual, and parotid glands, renal pelvis, ureter, and bladder. The metaplastic changes were as extensive in the rats of Group II on a diet deficient in vitamin A alone as in those of Group I which received a diet deficient in vitamins A and D.

CONCLUSION.

1. A dietary deficiency of vitamin A alone is adequate to induce metaplasia of columnar, cuboidal, and transitional epithelia to the squamous, keratinizing type in some organs.

2. Epithelial metaplasia to the squamous keratinizing type is of such frequency in young rats kept on a diet deficient in vitamin A for a long period of time (10 weeks or longer) as to constitute a good morphological indicator of the deficiency of this vitamin in the diet.

3. The metaplastic changes are as frequent and as great in rats on a diet deficient in vitamin A alone as in those on a diet deficient in vitamins A and D.

4. Inadequate food, provided the vitamins are supplied, does not induce the metaplasia.

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