rial. About 10 times the amount of antiserum to neutralize human thyrotropin was necessary to neutralize the effect of an approximately equivalent amount of LATS. Unfortunately this latter fact does not help distinguish among the speculations listed above as to the nature of LATS.

Summary. A globulin concentrate from antiserum to bovine pituitary thyrotropin completly neutralized the effect on the thyroid of "long-acting thyroid stimulator" in the serum of patients with Graves' disease. Both early and late responses in the McKenzie assay were abolished. A globulin concentrate from normal rabbit serum had no neutralizing effect. About 10 times as much antibody was required to neutralize LATS as the approximately equivalent potency of human pituitary thyrotropin.

1. Adams, D. D., Purves, H. D., *Proc. Univ. Otago Med. School*, 1956, v34, 11.
2. McKenzie, J. M., *Endrocrinology*, 1958, v63, 372.
3. Werner, S. C., Otero-Ruiz, E., Seegal, B. C., Bates, R. W., *Nature*, 1960, v185, 472.
4. Bates, R. W., Garrison, M. M., Howard, T. B., *Endocrinology*, 1959, v65, 7.
5. Werner, S. C., Seegal, B. C., Osserman, E., *J. Clin. Invest.*, 1961, v40, 92.
6. Howe, P. E., *J. Biol. Chem.*, 1921, v49, 93.
7. Kohn, J., *Nature*, 1959, v183, 1055.
8. McKenzie, J. M., Fishman, J., PROC. SOC. EXP. BIOL. & MED., 1960, v105, 126.

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Effect of Vitamin A Deficiency on Taste.† (27066)

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Vitamin A is necessary for maintaining the integrity of epithelial tissue. Since taste buds consist of modified epithelial cells, a role for vit. A in the taste mechanism readily suggests itself. It was recently reported that vit. A-deficient rats were apparently unable to taste quinine and saccharin(1). The present study explored this phenomenon using quinine sulphate and sodium chloride over an extended period of time, and tested the effect of administration of vit. A on recovery of normal taste function.

Materials and Methods. Eleven 6-week old albino rats (CFN, Carworth) were placed on the USP vit. A-deficient diet. Twenty four-hour, 2-choice preference trials began 14 weeks later, when the rats were still growing normally. The animals were in individual cages, fitted with 2 Richter-type graduated drinking tubes, one tube containing distilled water and the other, a test solution. Measurements were made to the nearest ml and the tubes were rinsed and filled with fresh solution every day. Percent preference was calculated as ml of test solution drunk, divided by ml of total fluid consumed, times 100. When total intake was less than 5 ml, the preference was not calculated. This criterion eliminated 13 readings out of 2800. A group of 9 normal rats received the same test solutions, and another group of 6 normal rats were given water in both tubes (H_2O control). The controls were subject to the same conditions as the experimentals. The right-left position of the tube containing the test solution was changed daily according to a restricted random schedule. Preference tests were divided into 8-day periods, during which each chemical was presented randomly for 4 days and appeared equally often in each position. The rats had a choice between either NaCl or quinine sulphate (QSO_4) and distilled water for 24 hours. Pre-

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ferences given in this paper are the group means for the 4 days.

Results. A decrease in degree of rejection of 8 μM QSO_4 solution occurred in the terminal stages of vit. A depletion (Fig. 1A). All the

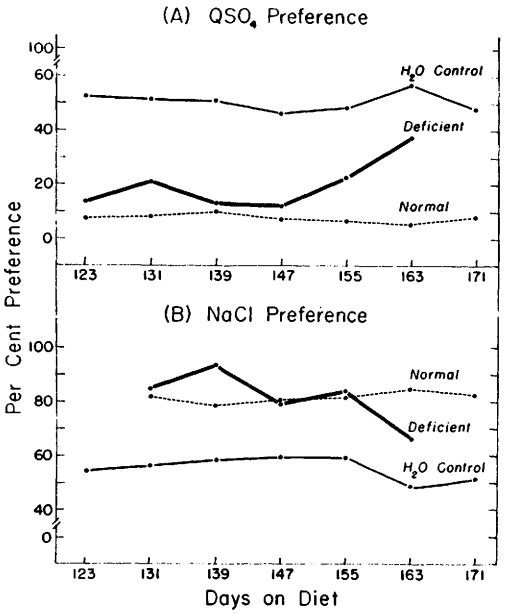

FIG. 1(A). Preference functions of normal and vit. A-deficient rats for 8 μM quinine sulphate (QSO_4) solution.

(B). Preference functions of the same rats for .05 (131 days), .02 (139 days), .01 (147 days,) and .008 M (155-163 days) NaCl solutions. Preference of H_2O controls is not significantly different in either case from an ideal 50% choice ($p=.71$; $p=.26$; 2-tailed).

deficient-group mean QSO_4 preferences, except those for 147 and 155 days on diet, are significantly greater than the normal group preferences for the 7 periods shown on the graph. P values range from < 0.0003 to 0.04; unless otherwise noted, this and subsequent p values are one-tailed and based on the Mann-Whitney U test(2).

Prior to 8 μM QSO, the rats were exposed to 40, 20, and 10 μM solutions, each for one full period. Significant differences appeared at 20 and 10 μM ($p<0.025$, $p<0.001$). At this time the rats were still growing normally. Weight losses began 16 days later, after 123 days on the diet.

Figure 1B shows the change in NaCl preference over the same 7 periods. Concentration was decreased in each period until 0.008 M was reached at 155 days. As was the case for quinine rejection, NaCl selection was significantly lower than the normals ($p<0.025$) toward the end of the depletion period. In fact, the deficient group was not significantly different from the H_2O controls ($p=0.08$). The deficient group showed a significantly higher preference for 0.02 M NaCl at 139 days ($p=0.002$, 2-tailed). The reason for the increased selection is not apparent.

Deaths occurred from secondary infections after 139 days on the diet. The last 3 means on both graphs represent 9, 7, and 5 rats respectively. During these 3 periods the deficient rats were regularly given antibiotics intramuscularly. Control rats were given an initial dose only. Four rats developed xerophthalmia and were given vit. A intraperitoneally. Two recovered.

Vit. A was given on an individual basis and the treated animals' preferences were not included with those of the non-treated. Preferences of the non-treated animals continued in the direction of indifference (50%), but because of the reduced group size, they are not plotted on the graphs.

The 2 surviving rats were studied for 6 and 8 weeks respectively after administration of vit. A. At the end of the period they had regained their weight and appeared normal. Table I shows that the treated rats differed significantly from the controls in QSO_4 preference. They did not differ significantly in preference for NaCl. Moreover, the normal NaCl preference was reestablished immediately following treatment. Mean preference of the rats in the 2 periods preceding vit. A treatment are listed for comparison.

TABLE I. Effect of Vit A on Mean Preferences of 2 Vit A Deficient Rats for QSO_4 and NaCl Solutions.

	QSO_4	NaCl
Pre-vit A	45.1	58.0
Post-vit A	47.2*	82.2‡
Control Rats	8.7	80.3

* $P<.003$ ‡ $P = .57$ (both two-tailed.)

Discussion. These results indicate that vit. A is required for normal taste function. Dowling and Wald(3) recently reported that rat liver stores and blood levels of vit. A dropped

to zero 2 to 3 weeks before weight losses began. At the same time, the rhodopsin level declined and the light threshold for the electroretinogram began to increase. The first significant change in QSO_4 rejection by our rats occurred in the third week preceding weight losses, which would correspond to the time when the supply of vit. A was completely depleted. However, since thresholds were not measured, changes may have occurred earlier. The late appearance of changes in NaCl selection and the lack of recovery of QSO_4 rejection suggests the possibility of different mechanisms underlying gustatory stimulation.

The rapid recovery of NaCl selection tends to rule out peripheral nerve damage or physical blocking of the taste pore as the reason for the taste deficit. A direct effect on the taste cells may reasonably be hypothesized. Vit. A may thus play a role in the functional and/or structural integrity of the taste cells. Verification will require histological and electrophysiological studies.

Further, the effect of vit. A may be specific or merely the result of a general effect on epithelial tissue. Dowling and Wald(4) reported that vit. A acid maintained normal growth in rats but did not prevent development of night blindness, thus limiting the role of vit. A to the visual system. An experiment is now underway in our laboratory to determine whether vit. A acid may also fail to prevent development of a taste deficit. If this is the case, vit. A would be implicated as a specific requirement in taste as in vision.

Summary. Rats in the advanced stages of vit. A depletion showed abnormal taste responses. A marked decrease in rejection of quinine and selection of NaCl solutions occurred. Administration of vit. A resulted in rapid recovery of NaCl selection but not of quinine rejection. These data suggest that vit. A may have a direct effect on the functioning of the taste cells.

1. Seifter, E., Rothberg, H., Berkman, J. I., *Abst. of Papers*, 1960, Am. Chem. Soc. 137th Meeting, 17N.

2. Siegel, S., *Non-Parametric Statistics*, 1956, McGraw Hill, New York.

3. Dowling, J. E., Wald, G., *Proc. Nat. Acad. Sci. U. S.*, 1958, v44, 648.

4. ————, Wald, G., *ibid.*, 1960, v46, 587.

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Effect of Estrogen and Estrogen-like Compounds on Atherosclerosis in Cholesterol Fed Cockerels. (27067)

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Exogenous sex hormones have been observed to modify coronary atherosclerosis in experimental animals(1). Feminizing doses of estradiol benzoate will inhibit coronary atherosclerosis in cholesterol fed cockerels and will induce regression of pre-existing lesions. The mechanism of estrogen inhibition of coronary atherosclerosis is as yet unclear, though data suggest a relationship with its feminizing potency(2). Three estrogen-like compounds of low feminizing potency,* synthesized and purified by Dr. Paul Keurath, were used to study further the relationship between these effects. Two of these analogues did have an effect on the course of the experimental disease.

Methods. Forty white leghorn cockerels, one month of age, were divided into 6 experimental groups, and each bird was allocated to an individual cage. All birds received a basic diet of standard chick growing mash. The experimental diet was formulated by supplementing the growing mash with 1% cholesterol and 20% cottonseed oil. The 3 estrogen-like analogues, 2 iodo-estradiol, 2 iodo-estrone and 2 iodo-estrone acetate, all of low

* Feminizing effects of these compounds were bioassayed in ovariectomized rats by the Endocrine Laboratories, Madison, Wisc.