the temperature was well within the range observed in uninoculated mice.

Summary and Conclusions. 1. Hypothermia rather than fever was the characteristic thermal response of mice to intranasal inoculation of the PR8 strain of type A influenza virus. 2. Mice showed marked hypothermia with fatal termination in response to inoculation of large amounts, e.g., 1,000 or more infectious doses (ID_{50}), of 2 sublines of the virus, one adapted to embryonated eggs and the other to mice. 3. Inoculation of mice with a small amount of virus, e.g., one to two ID_{50}, did not cause hypothermia. In mice inoculated with about 2 ID_{50} of egg-line virus, the maximum level of virus in the lungs was lower than in mice inoculated with larger amounts of virus, and pulmonary lesions were present but failed to reach the level of 50% total lung-lesion score. 4. In mice inoculated with a moderate amount of egg-line virus, e.g., about 200 ID_{50}, the maximum virus level in the lungs was reached 3 to 4 days before the occurrence of hypothermia. Time of occurrence and degree of hypothermia appeared to be closely related to the extent of pulmonary lesions. 5. The mouse-passaged virus caused more rapid development of hypothermia than the egg-line virus. 6. It is concluded that hypothermia is related to the extent of pulmonary lesion rather than to the extent of virus increase *per se*.

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Neutralization of "Long-Acting Thyroid Stimulator" of Graves' Disease by Antisera to Bovine Pituitary Thyrotropin.* (27065)

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Adams and Purves(1) assayed the serum of patients with Graves' disease for thyrotropin, in guinea pigs given I-131 to label the thyroid and then thyroxine to suppress endogenous release of thyrotropin. They observed the test animal's response to patients' serum to be different from that to standard thyrotropin or to serum from patients with myxedema. Injection of standard thyrotropin or of myxedema serum increased the circulating level of I-131 and a maximum value was reached after a few hours. The level then decreased and was significantly lessened by 16 hours. The serum from Graves' disease on the other hand caused little increase in level early after injection but produced a maximal response at 16 hours or later.

These findings have been amply confirmed in the mouse, similarly prepared for assay purposes except that the responses can be measured at 2 hours and 9 hours after injection(2). The term "long-acting thyroid

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stimulator" (LATS) has recently replaced "serum factor" and "abnormal activator", originally proposed to describe the thyrotropin-like substance in the serum.

Werner and co-workers investigated immunochemically(3) whether LATS is similar or not to human pituitary thyrotropin. Using an antiserum to bovine pituitary thyrotropin, they were able to neutralize the effect on the thyroid of both a human thyrotropin preparation and of a preparation of patients' serum known to contain LATS, made by the alcohol percolation method(4). However, the prepared serum gave only the 2 hour response in the McKenzie mouse assay, so neutralization of the late 9 hour response could not be tested. McKenzie and Fishman(8) then attempted to neutralize the 9 hour response produced by injection of untreated serum from patients with Graves' disease but were unable to do so in 4 trials, although succeeding in neutralizing the 2 hour response to the thyrotropin present in serum from patients with myxedema. They concluded that the serum factor in Graves' disease was not pituitary thyrotropin.

Werner and collaborators had had to use a concentrated globulin from the antiserum to thyrotropin in order to obtain sufficient concentration of antibody to achieve neutralization of LATS, whereas McKenzie and Fishman had used unconcentrated antiserum. It thus appeared likely that the failure of these latter workers could have been due to use of insufficient antibody rather than that LATS was not thyrotropin. Accordingly the problem was reinvestigated except that a globulin concentrate of anti-bovine thyrotropin serum was used as the source of antibody.

Methods. Thyrotropin hormone preparations. The bovine thyrotropin preparation used both as an antigen and as a thyrotropin standard is one in clinical use (Thytropar, Armour). It had a potency of approximately 1 i.u./mg. The human pituitary thyrotropin preparation employed was a relatively crude one made available by Drs. John Bakke and Lawrence Heidemann(3).

Preparation of antisera. Antiserum was developed in rabbits by the methods already reported(5). Six animals were immunized with bovine thyrotropin. An average of 4 i.u. of the thyrotropin was incorporated in 1.0 ml of Freund's adjuvant and injected subcutaneously. Intravenous booster injections were also given until a total of 36 i.u. was given to each rabbit over a period of 2½ months. Ten days after final injection, the animals were exsanguinated under sterile conditions and the sera stored without preservative at 4°C. Globulin fractions of normal serum and antiserum were prepared by sodium sulfate precipitation(6). The volume of the aqueous solutions was reduced to the desired level by dialysis against polyethylene glycol(7), to limit the volume injected intravenously into the test mice.

Bioassay methods. The assay method of McKenzie(2) was employed(3,5) and the tests conducted on the fifth day after I-131 and thyroxine administration. Control blood samples were obtained and the test materials injected intravenously. At 2 and 9 hours after injection, blood samples were again obtained. Radioactivity in the blood samples was measured in an automatic Packard well-type scintillation counter with an average background count of 20 cpm. Groups of 4 mice were employed for each test point. Testing for neutralization of thyrotropin activity was conducted according to reported procedure(5). Antiserum, or the globulin fraction, was mixed with the thyrotropin preparation or serum to be neutralized, placed at 4°C overnight, and then injected without centrifugation the following morning. Since previous absorption of the antiserum with bovine serum to remove anti-bovine serum proteins did not affect the neutralizing potency of the antiserum, absorption was not performed routinely.

Results. Neutralization of beef and human pituitary thyrotropin preparations. The amounts of antiserum required for complete neutralization of both bovine and human pituitary thyrotropin preparations was determined. The findings are summarized in Table 1. Complete neutralization of 0.1 mu bovine thyrotropin preparation was achieved with 0.1 cc of anti-bovine thyrotropin serum, whereas with 0.1 mu human thyrotropin preparation, 0.25 cc of the same antiserum or of an equivalent amount of antiserum globulin was required. Control experiments showed no neutralization by normal rabbit serum or globulin of thyrotropin.

TABLE I. Comparative amounts of antiserum required to neutralize completely bovine and human thyrotropin.

Exp. No.	Test Material	No. of mice	Dose: Thyrotropin or patient serum mu	Dose: Rabbit serum or serum equiv. cc	Blood radioactivity (% of preinjection value as 100%) 2 hr Mean ± SD	Blood radioactivity (% of preinjection value as 100%) 9 hr Mean ± SD	P value of difference from value with normal serum or globulin
383	Saline	4	—	—	84 ± 14	73 ± 19	—
	Bovine thyrotropin (ATSH)	4	.1	—	278 ± 31	194 ± 29	—
	ATSH + Anti-serum	4	.1	.1	133 ± 26	119 ± 31	<.001
	idem	4	.1	.25	125 ± 30	150 ± 23	<.001
397	Saline	4	—	—	124 ± 11	92 ± 31	—
	Human thyrotropin (HTSH)	4	.1	—	247 ± 10	180 ± 17	—
	HTSH + Normal rabbit serum	4	.1	.5	212 ± 60	151 ± 42	—
	HTSH + Anti-serum	4	.1	.1	190 ± 32	155 ± 18	>.1
	idem	4	.1	.25	134 ± 20	145 ± 13	<.01
	idem	4	.1	.5	129 ± 19	107 ± 12	<.001
403	Saline	4	—	—	104 ± 26	81 ± 17	—
	Human thyrotropin	4	.1	—	217 ± 23	122 ± 24	—
	HTSH + Normal Rabbit globulin	4	.1	.25	233 ± 11	188 ± 44	—
	HTSH + Anti-globulin	4	.1	.3	147 ± 22	89 ± 22	<.001
	idem	4	.1	.75	149 ± 9	80 ± 3	<.001

TABLE II. Partial neutralization of Graves' disease serum factor by small amounts of anti-bovine thyrotropin serum.

Exp. No.	Patient	Test Material	No. of mice	Dose: Thyrotropin or patient serum	Dose: Rabbit serum or serum equiv. cc	Blood radioactivity (% of preinjection value as 100%) 2 hr Mean ± SD	Blood radioactivity (% of preinjection value as 100%) 9 hr Mean ± SD	P value of difference from value with normal serum or globulin
387	L.L.	Saline	4	—	—	93 ± 24	92 ± 18	—
		Bovine thyrotropin	4	.1mu	—	228 ± 22	177 ± 86	—
		Serum	4	.1cc	—	261 ± 36	536 ± 41	—
		Serum + Normal Rabbit serum	4	.1 "	.4	302 ± 38	632 ± 147	—
		Serum + Anti-serum	4	.1 "	.4	329 ± 41	519 ± 34	<.1
294	R.H.	Saline	4	—	—	78 ± 12	79 ± 9	—
		Bovine thyrotropin	4	.1mu	—	164 ± 44	151 ± 56	—
		Serum	4	.25cc	—	70 ± 20	241 ± 12	—
		Serum + Normal Rabbit globulin	4	.25 "	1.0	171 ± 26	286 ± 36	—
		Serum + Anti-serum	4	.25 "	1.0	122 ± 47	171 ± 29	<.001
334	R.H.	Saline	4	—	—	105 ± 27	136 ± 21	—
		Bovine thyrotropin	4	.5mu	—	226 ± 9	171 ± 5	—
		Serum	4	.5cc	—	141 ± 5	211 ± 18	—
		Serum + Normal Rabbit globulin	4	.5 "	1.0	326 ± 49	463 ± 66	—
		Serum + Anti-globulin serum	4	.5 "	1.0	281 ± 74	338 ± 50	<.001
420	R.H.	Saline	4	—	—	100 ± 13	100 ± 13	—
		Human thyrotropin	4	.1mu	—	343 ± 73	286 ± 64	—
		Serum	4	.2cc	—	248 ± 44	364 ± 109	—
		Serum + Normal Rabbit globulin	4	.2 "	1.0	282 ± 128	343 ± 152	—
		Serum + Anti-globulin	4	.2 "	1.2	183 ± 20	240 ± 53	<.1

Neutralization of LATS of Graves' disease. Ten experiments were conducted with serum from each of 6 patients with Graves' disease (Tables 2 and 3). Five of the 6 had severe eye changes, the sixth had no ocular manifestations. Amounts of antisera or corresponding amounts of globulin employed varied from 0.4 cc to 2.5 cc per mouse. These were mixed before assay with the minimum amount of patient's serum capable of giving an unequivocal 9 hour assay response. Control studies were made concurrently.

TABLE III. Complete neutralization of Graves' disease serum factor by large amounts of antibovine thyrotropin serum.

Exp. No.	Patient	Test Material	No. of mice	Dose: Thyrotropin or patient serum	Dose: Rabbit serum or serum equiv. cc	Blood radioactivity (% of preinjection value as 100%) 2 hr Mean ± SD	9 hr Mean ± SD	P value of difference from value with normal serum or globulin
452	G.C.	Saline	4	—	—	124 ± 10	123 ± 27	—
		Bovine thyrotropin	4	.1mu	—	328 ± 15	251 ± 39	—
		Serum	4	.1cc	—	174 ± 42	311 ± 63	—
		Serum + Normal Rabbit globulin	4	.1 "	2.0	177 ± 9	269 ± 73	—
		Serum + Anti-globulin (unabsor)	4	.1 "	2.0	147 ± 34	132 ± 18	<.001
		Serum + Anti-globulin (absor)	4	.1 "	2.0	132 ± 4	116 ± 7	<.001
438	L.W.	Saline	4	—	—	106 ± 10	87 ± 10	—
		Bovine thyrotropin	4	.1mu	—	189 ± 8	155 ± 18	—
		Serum	4	.05cc	—	171 ± 53	223 ± 129	—
		Serum + Normal Rabbit globulin	4	.05 "	2.5	131 ± 16	219 ± 43	—
		Serum + Anti-globulin	4	.05 "	2.5	123 ± 18	134 ± 18	<.001
434	B.J.	Saline	4	—	—	85 ± 31	83 ± 16	—
		Bovine thyrotropin	4	.1mu	—	386 ± 81	238 ± 86	—
		Serum	4	.1cc	—	151 ± 14	214 ± 34	—
		Serum + Normal Rabbit globulin	4	.1 "	2.5	175 ± 34	217 ± 24	—
		Serum + Anti-globulin	4	.1 "	2.5	142 ± 42	145 ± 67	.01
419	B.J.	Saline	4	—	—	80 ± 8	63 ± 23	—
		Human thyrotropin	4	.1mu	—	261 ± 31	190 ± 17	—
		Serum	4	.1cc	—	124 ± 16	201 ± 27	—
		Serum + Normal Rabbit globulin	4	.1 "	2.5	148 ± 12	211 ± 22	—
		Serum + Anti-globulin	4	.1 "	2.5	121 ± 34	149 ± 41	<.001
413	L.L.	Saline	4	—	—	113 ± 5	110 ± 17	—
		Bovine thyrotropin	4	.1mu	—	240 ± 34	194 ± 30	—
		Serum	4	.05cc	—	134 ± 15	199 ± 26	—
		Serum + Normal Rabbit globulin	4	.05 "	2.5	157 ± 34	214 ± 47	—
		Serum + Anti-globulin	4	.05 "	2.5	122 ± 23	124 ± 22	<.001

Inadequate amounts of antiserum gave partial neutralization of the LATS in patients' sera (Table 2). However, complete neutralization was achieved with larger amounts of antiserum in all of 5 experiments, and the I-131 level in the serum of the test mice failed to rise significantly above pre-injection value at either 2 or 9 hours after injection (Table 3). Specificity of the neutralization reaction was confirmed by the demonstration that neither preliminary absorption of the antiserum with bovine serum nor incubation with normal rabbit serum globulin caused neutralization of LATS.

Discussion. The successful neutralization of LATS in the serum of patients with Graves' disease accomplished with antibody to bovine thyrotropin confirms the previous findings of this laboratory(3). To achieve neutralization of a minimally effective amount of LATS, antibody from 2.5 cc of antiserum was required. This amount is far in excess of that used by McKenzie and Fishman(8) and undoubtedly explains their failure.

The ability of antiserum to thyrotropin to neutralize LATS suggests several possibilities as to its nature: 1) that it is circulating human pituitary thyrotropin with another substance or substances in the patients' serum accounting for both the long-acting effect and the large doses of antibody needed for neutralization; 2) that it is circulating human pituitary thyrotropin but is bound differently to carrier proteins than in the normal state; or 3) that it is different from human pituitary thyrotropin but has sufficient determinants in common so that it can cross-react with, and be neutralized by, antithyrotropin sera.

About 2.5 times as much anti-bovine thyrotropin serum was required to neutralize the same amount of standard human pituitary preparation as standard bovine hormone mate-

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.

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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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