in cell culture materials. A second possibility is that the inactivation procedures used may have destroyed antigenicity as well as infectivity. It is also possible that along with the well recognized alteration in virulence of herpes simplex virus on egg passage, there is some accompanying change in antigenic potency. In fact, a recent report indicates that herpes virus grown in eggs may be less active antigenically than virus from cell culture or mouse brain even if pre-inactivation titers are comparable(10).

It is clear from our experiments and those of Chu and Warren(10) that injection of inactivated herpes simplex virus in mice can protect against a large intracerebral challenge. Production of demonstrable neutralizing antibody also results from these injections.

Summary. Ultraviolet inactivated herpes simplex virus prepared in rabbit kidney cell cultures was injected intravenously and intraperitoneally into mice. The majority of mice so immunized survived a 1000 LD_{50} intracerebral herpes simplex virus challenge and were shown to possess neutralizing antibody against the virus. These findings are contrary to the findings of studies in which inactivated allantoic fluid virus was used as antigen. However, they are in agreement with early experiments which employed inactivated herpes simplex virus prepared in mouse or rabbit brain and a recent report in which antigen was also prepared in rabbit kidney cell culture.

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Effect of Hyperthyroidism on Body Weight Gain and Feed Consumption in Male and Female Rats.* (26674)

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Normal thyroxine secretion rates (TSR) of many female rats of Sprague-Dawley-Rolfsmeyer (S-D-R) strain have been determined in this laboratory(1,2). TSR varied from 0.25 μg to 2.0 μg/100 g B W, with mean of about 1.0 μg. A significant increase in TSR was observed in lactating rats(3). Lactating rats showed a range up to 3.0 μg. Use of 3.0 μg/100 g B W has been shown to stimulate significant increases in mammary gland growth(4,5) and lactation(6). Unpublished data on TSR of normal male rats of this strain show a range of 0.5 μg to 1.5 μg/100 g B W with a mean of about 1.0 μg/100 g B W.

The object of the present experiment was to relate mean and maximum TSR of this strain of rats with optimal level of hyperthyroidism for weight gain. It was considered that injection of graded levels of thyroxine above mean and maximum TSR would be reflected in changing weight gain in such a manner as to indicate optimal hyperthyroidism and transition to a state of thyroxine induced stress.

Material and methods. Male and female

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TABLE I. Influence of Increasing Levels of Thyroxine on Body Weight Gains of Male and Female Rats.

Thyroxine level (μg/100 g BW)	Start	Days of treatment: 20	40	80	120	160	200
		Avg body wt (g)					
Control ♂	(7)*45	(7) 160.7	(7) 274.4	(6) 387.8	(5) 448.8	(5) 472.4	(5) 506.6
♀	(11) 44	(11) 137.8	(10) 192.2	(10) 248.2	(10) 265.2	(10) 271.3	(10) 279.9
3.0 μg ♂	(7) 44	(7) 160.7	(7) 257.2	(4) 341.0	(4) 378.5	(4) 396.5	(4) 398.0
♀	(11) 38	(10) 112.7	(9) 181.1	(9) 228.4	(9) 242.8	(9) 260.2	(9) 266.2
6.0 μg ♂	(9) 37	(9) 154.2	(9) 248.7	(9) 314.0	(7) 328.8	(8) 317.6	(5) 308.0
♀	(9) 38	(8) 131.0	(8) 187.5	(7) 222.0	(7) 249.0	(7) 263.0	(7) 271.8
9.0 μg ♂	(9) 45	(7) 153.8	(7) 241.5	(6) 301.5	(6) 321.5	(6) 345.5	(5) 362.0
♀	(9) 44	(9) 124.4	(9) 185.5	(9) 224.1	(9) 239.0	(8) 245.8	(4) 260.7
12.0 μg ♂	(9) 51	(9) 152.5	(9) 233.1	(7) 297.7	(6) 319.0	(3) 342.2	(3) 331.5
♀	(9) 47	(9) 133.1	(9) 191.5	(9) 227.4	(9) 247.7	(9) 253.7	(9) 263.3

* No. of animals in parentheses.

rats of Sprague-Dawley-Rolfsmeyer (S-D-R) strain, age 26 days, were injected daily with 3.0 μg, 6.0 μg, 9.0 μg, and 12.0 μg L, thyroxine/100 g body weight for 200 days. Control group was injected with an alkaline saline solution. Body weight was recorded at regular intervals. Commercial lab feed (Purina) was supplied *ad libitum*. Animal room was maintained at uniform temperature of 78 ± 1°F.

When animals were mature, accurate feed consumption records were kept for a period of 12 days. Daily feed consumption was expressed as g/100 g B W.

Results. In relation to normal thyroxine secretion rate in females, thyroxine (T_4) at 3 μg/100 g B W is about 3 times mean secretion of this strain and about ⅓ above maximum secretion rate. In comparison to lactating rats, 3 μg T_4 is equal to maximal level observed.

Female animals injected with 3 μg T_4 weighed 82% of controls at 20 days, and 94, 92, 92, 96 and 95% of the controls at successive 40-day intervals up to 200 days (Table I). In successive groups of females, body weights were reduced no more than 10% below the controls in any age group. As level of T_4 was increased no trend toward greater weight reduction was observed.

In males, injection of 3 μg T_4 began to influence body weight gains at 40 days, and continued to 200 days with a reduction to 79% in comparison to controls at that time. Similarly, at 6 μg T_4 weight reduction was increased to 61% of controls, at 9 μg to 71%, and at 12 μg to 65% of the controls. Thus, male rats were more severely rstricted in body weights by progressive degrees of hyperthyroidism than were females. Further, females survived the treatment much better than did males.

Mean food consumption per 100 g B W of control animals was 4.9 g for males and 5.4 g for females. As T_4 level increased, food consumption progressively increased by 37% in females and over 70% in males.

Discussion. In past experiments, physiological effects of thyroxine administration have been reported in many types of experimental animals. In many cases, amount of thyroxine injected was excessive in relation to recently observed normal TSR and question could be raised whether results indicated physiological effect of thyroxine or a stress-induced effect. With development of a quantitative method of estimating TSR and availability of data indicating mean and

TABLE II. Influence of Increasing Levels of Thyroxine upon Mean Daily Feed Intake.

Thyroxine level (μg/100 g BW)	Mean daily feed consumption (per 100 g body wt) ♂ g	♂ %*	♀ g	♀ %*
Control	4.9		5.4	
3.0	5.6	14.2	6.0	11.1
6.0	7.2	46.9	6.5	20.3
9.0	8.5	73.4	7.2	33.3
12.0	8.4	71.4	7.4	37.0

* % increase over controls.

range of normal TSR of both sexes in S-D-R strain of rats, it seemed desirable to determine optimal range of hyperthyroidism (in relation to mean and maximum TSR) and levels where stressful effects begin to appear.

In previous studies it was shown that 3 μg T_4/100 g B W significantly stimulated growth of mammary gland(4) and intensity of milk secretion(6) in this strain. This level was considered optimal for these physiological processes in mature animals. In present study, it is shown that this level depressed body weight gains of females temporarily, but weight was largely regained at later age intervals. More surprising, however, was the observation that 2, 3, or 4 times this level of T_4 had no greater effect upon depression of body weight. This indicates that in females up to 12 times mean TSR level is well tolerated by growing rats. It indicates, also, that mild hyperthyroidism has no stimulating effect upon rate of weight gain.

In Long-Evans strain of rats, Evans *et al.* (7) noted a differential in biological effectiveness of thyroxine after thyroidectomy. Nearly normal growth was supported in females at 0.25 μg and at 0.50 μg growth was within normal limits.

In male rats, effect of T_4 was minimal during first 20 days of administration: thereafter its depressing effect upon body weight was progressive until end of experiment. Level of T_4 of 6 to 12 μg/100 g B W was quite similar in its depressing effect upon body weight. These levels depressed body weight far more in males than in females.

Present results are in general agreement with Koger and Turner(8) who observed depressions in body weights of male and female rats following oral administration of increasing levels of thyroprotein to S-D-R strain. Nose-anus length of males was slightly greater than controls in spite of lower body weights.

Causes of differences in weight gain of 2 sexes is obscure. It has been suggested that TSR might play a role. Data indicate that mean TSR of 2 sexes of normal animals is similar. Further, levels of T_4 above normal are without benefit on weight gain of females. In males, higher levels of T_4 tend to depress body weight more severely than in females. Clearly, in rats, T_4 plays no role in sex difference in rate of gain nor in mature body weight.

It has long been recognized that hyperthyroidism stimulates appetite and increases feed intake. Present data confirm that observation. Not recognized, heretofore, is sex difference. Females were capable of maintaining more nearly normal weight gain on higher levels of T_4 yet consumed less additional feed than did males.

It is possible that sex differences exist in regard to metabolism of T_4. In cows it has been shown that $t\frac{1}{2}$ of T_4 is reduced about 54% when T_4 was injected 50% above TSR (9). Observed differences in sexes in present study might be explained, in part, if $t\frac{1}{2}$ of T_4 in hyperthyroidism is shown to differ in sexes of rats.

No evidence of a T_4 induced stress was observed in females of this strain when up to 12 times mean TSR was administered. In males, levels of 6 times or more severely restricted body weights with advancing age.

Summary. Male and female rats of Sprague-Dawley-Rolfsmeyer strain, age 26 days, were injected daily with 3.0, 6.0, 9.0, and 12.0 μg L, thyroxine/100 g B W for 200 days in comparison with normal groups. Lowest level was 3 $\times$ mean TSR and equal to highest rate observed in lactating rats. In females, weight gain was reduced by less than 10% in comparison with controls with increasing age, and no trend toward greater weight restriction was observed on higher levels of T_4. In males, increased weight restriction appeared at 40 days and beyond, and was more marked than in females. Levels of 6 μg and above permitted gain equal to 60 to 70% of that of controls. Food consumption increased progressively on higher T_4 levels but females showed less increase than did males.

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Determination of Alpha-glycerophosphate Oxidation with Tetrazolium.* (26675)

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A variety of technics are presently employed for assay of alpha-glycerophosphate dehydrogenases, but none are entirely satisfactory in terms of precision or reproducibility. Growing emphasis on the significance of glycerophosphate oxidation in muscle metabolism(1), endocrine dysfunction(2), and the biochemical characteristics of tumors(3) indicates the need for a simple and reliable technic for assaying the enzyme systems involved in oxidation of glycerophosphate. INT (2-p-iodo-3-p-nitro-5-phenyl tetrazolium chloride), a monotetrazolium salt, has been used successfully for measuring a number of enzyme systems(4,5). The present communication describes and evaluates a simplified technic utilizing INT for determination of the activities of the soluble (DPN-dependent) and insoluble (cytochrome-dependent) alpha-glycerophosphate oxidase complex (AGPO) and the corresponding specific alpha-glycerophosphate dehydrogenases (AGPD) in homogenates of animal tissues.

Method. Excised tissues of V stock house mice (Bar Harbor) were placed in iced Ringer solution, and homogenates of appropriate strengths made up with deionized distilled water. Homogenization was carried out with motor-driven teflon pestles in glass tubes. Total AGPO was assayed in 10 ml test tubes containing 0.2 ml of 0.5M AGP (alpha-glycerophosphate, Sigma), 0.3 ml of 0.4% INT (Dajac), 0.2 ml of 1% DPN (Sigma), and 0.2 ml of 0.5% or 1.0% homogenate. M/15 phosphate buffer (pH 8.0) was added to bring the total volume to 2.0 ml. In assays for insoluble AGPO, DPN was omitted. Corresponding assays for total and insoluble AGPD were carried out by adding 0.2 ml of 10^{-4}M phenazine methosulfate (Sigma) and 0.2 ml of 10^{-3}M NaCN to reaction mixtures before bringing to a final volume of 2.0 ml. This step establishes direct electron transfer between the dehydrogenase and INT. Appropriate blanks containing no substrate were run in all experiments. After uniform agitation (by holding against a rotating cuboidal rubber stopper for 10 seconds) the tubes were placed in a water bath at 37°C for 15 minutes. The reaction was stopped with 0.2 ml of 30% TCA. Prolonged contact with the acid should be avoided, as a 5% loss of formazan was found after 24 hours exposure to the TCA. The iodoformazan (IF) produced during incubation was extracted with 5 ml ethyl acetate, shaken vigorously 50 times, centrifuged, and the decanted ethyl acetate extract read spectrophotometrically (Coleman Jr.) at 490 mμ against a calibration curve of chemically reduced (hydrosulfite) IF in ethyl acetate. Total nitrogen values in equivalent aliquots of homogenate were determined by the micro-Kjeldahl method and enzyme activities expressed as μg IF per μg tissue N. Values for DPN-dependent enzyme activity were obtained by subtracting the values for the in-

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