Effect of heat stress and DHT on thyrotropin (TSH) and thyroxin (T-4) serum level

Group No.	Treatment DHT and temperature	Body wt. on day of start (g)	Body wt. on day of end (g)	TSH levels of serum (mU/ml)	T-4 levels of serum (μg/100 ml)	Total plasma Ca^{++} (mg/100 ml)	Mortality (%)
1	Control 22 °C	44 ± 5	120 ± 6	1.0 ± 0.04	2.5 ± 0.1	10.3 ± 0.1	0
2	DHT i.p. 22 °C 500 μg/kg/day	45 ± 5	125 ± 5	2.1 ± 0.06	5.94 ± 0.029[a]	10.6 ± 0.1	0
3	Control 34 °C	45 ± 5	70 ± 10	1.3 ± 0.2	1.1 ± 0.03[a]	10.5 ± 0.2	60[a]
4	DHT i.p. 34 °C 500 μg/kg/day	45 ± 5	110 ± 5	1.9 ± 0.3	2.3 ± 0.12[a]	10.5 ± 0.1	0
5	Control 37 °C	45 ± 5	47 ± 5	0.1 ± 0.2[a]	0.42 ± 0.02[a]	10.4 ± 0.2	100[a]
6	DHT i.p. 37 °C 500 μg/kg/day	45 ± 5	50 ± 3	0.5 ± 0.1[a]	0.92 ± 0.05[a]	10.5 ± 0.1	0

Each of the 6 groups consisted of 12 male rats. Group 1–4 was 21 days old when treatment started and 42 days old when stopped. Group 5-6 was 21 days old when treatment started and had to be killed after 4 days because of imminent death of the control group. (Means ± S.E.M.). [a] Significant ($P < 0.001$).

BALOGH et al.[6] and BAJUSZ[7] found high BMR levels in rats kept at temperatures above 33 °C. Our findings of low T-4 levels at high temperatures may explain the high mortality at these temperatures, as the rats cannot then cope with their high metabolic rate while lacking enough T-4 for negative feedback. It is, therefore, suggested that a certain optimal level of T-4 is a prerequisite for survival of animals at high temperatures. This view is also based on the rather high T-4 levels in the control rats kept at 34 °C (group 3), which could adapt themselves to heat, whereas the control rats kept at 37 °C (group 5) died early showing extremely low T-4 levels. No significant difference could be detected between the TSH levels at 22 °C and 34 °C (groups 1 and 3), while a highly significant drop in plasma TSH was noticed at 37 °C (group 5) as compared with the former. This drop in TSH at 37 °C was partly set off by DHT injections (group 6). The quantities of DHT injected in this experiment (500 μg/kg/day) did not affect plasma Ca levels. Still, DHT increased both TSH and thyroxine levels at all 3 experimental temperatures tested. This increase was significant in spite of the steep drop in blood TSH and thyroxine obtained at 37 °C. This shows that the thyroxine-TSH negative feedback is partly impaired by an ambient heat of 34 °C and completely abolished at 37 °C.

Zusammenfassung. Dehydrotachysterol (DHT 500 μg/kg/24 h) erhöht den TSH- und T-4-Spiegel in Rattenserum bei normalen und hohen Temperaturen, ohne den Calcium-Spiegel zu verändern, und verhindert den Tod von Ratten, die bei 34° oder 37 °C gehalten werden. Das Überleben der Ratten nach DHT-Injektionen erklärt sich durch erhöhte T-4-Produktion, die einen negativen «Feedback» auf den Hypothalamus ausübt und so die lethale Überproduktion von TSH verhindert.

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Effect of Decreased Dietary Protein upon Mammary Gland Growth in Rats

Experimental and clinical studies have shown that chronic and acute starvation, caloric restriction, vitamin and mineral deficiencies alter the function of endocrine glands. Restricted food intake resulted in reduced activity of the thyroid gland[1,2]. SINGH et al[3]. reported that decreased dietary protein, i.e., protein-free died and 5% protein diet, reduced thyroid hormone secretion rate (TSR) in rats. It has also been reported that absence of dietary protein caused decrease in FSH secretion which eventually affects estrogen and progesterone secretion. Several workers[4–6] have shown that thyroid hormone administered in intact rats or ovariectomized rats treated with estrogen and progesterone increased the amount of mammary gland growth. Estrogen, progesterone and prolactin are the main hormones essentially required for mammary gland development. In the present study, the effect of decreased dietary protein upon mammary gland growth by DNA estimation has been studied.

Materials and methods. 106 virgin female rats (approximately 70 days old) of the Sprague-Dawley Rolfsmeyer strain (purchased from Rolfsmeyer Co., Madison, Wis-

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Effect of decreased dietary protein upon mammary gland growth

Groups	Treatment	No. of animals	Body weight (g) Initial	Final	Dry fat-free Tissue ± SE (mg)	DNA/mg DFFT Mean ± SE (μg)	Total DNA Mean ± SE (mg)	Change over control Total DNA (%)
1	Control	20	242	266	967.4 ± 62.1	19.8 ± 0.5	19.0 ± 1.1	
2	Protein-free diet	17	256	187	480.2 ± 36.2	32.9 ± 0.5	15.1 ± 0.9[a]	–20.5
3	Protein (5%)	19	237	217	591.6 ± 40.3	27.6 ± 1.7	16.1 ± 1.3[b]	–15.3
4	Protein (10%)	18	244	246	745.5 ± 40.1	26.8 ± 1.6	19.4 ± 1.1	2.1
5	Protein (15%)	16	258	267	782.7 ± 5.2	25.6 ± 1.8	18.8 ± 0.6	– 1.1
6	Protein (20%)	16	241	261	849.1 ± 35.8	22.8 ± 1.1	19.2 ± 1.1	1.0

Significance from control using Student's t-test. DFFT, dry fat-free tissue. SE, standard error. [a] $P < 0.001$. [b] $P < 0.05$.

consin) were maintained under a uniform temperature (25 ± 0.5 °C) with 14 h light and 10 h darkness. Rats were divided randomly in 6 groups, 16–20 in each group (Table). Rats were maintained on graded level of protein; 1. Purina Lab chow (23.4% protein), Control group; 2. protein-free diet; 3. 5% protein diet; 4. 10% protein diet; 5. 15% protein diet; and 6. 20% protein diet. They were given water ad libitum. Details of composition of diet has been described elsewhere[3]. The rats were ovariectomized and were allowed 14 days for recovery, then were injected with 2.0 μg estradiol-17β + 6.0 mg progesterone dissolved in 0.5 ml sesame oil daily for 19 days. The protein diet was continued during this period. They were sacrificed by decapitation and 6 abdominal-inguinal mammary glands were removed and placed in separate beakers in the deep-freeze at –20 °C for at least a week to reduce all activitities of the enzyme, deoxyribonuclease. The glands were extracted in 95% alcohol for 8 h and then re-extracted with fresh alcohol for 6 h. The glands extracted with alcohol were then extracted with ether for 8 h. Dry fat-free tissue (DFFT) was weighed on a precision balance. The balance was sensitive to 0.01 mg. DNA was determined by the method of Webb and Levy[7] using highly polymerized DNA as a standard (Nutritional Biochemical Corp.). The assumption here is that the cells are mononuclear and the DNA is indicative of number of cells. The data were analyzed by Student's t-test.

Results and discussion. The mean total DNA of the group receiving a protein-free diet and 5% protein diet differed significantly ($P < 0.001$, $P < 0.05$, respectively) as compared to the control group. With increase in percentage of protein in the diet, the mammary gland growth was not reduced as indicated by DNA estimation. No decrease in body weight was observed when 10% or more protein was fed in the diet (Table). If the reduction in DFFT in the protein-free diet was due solely to loss of cells, then the DNA/mg DFFT should be the same as the control; however, it is decreased significantly ($P < 0.001$) suggesting that in addition there was loss of non-DNA cellular material. With increasing protein in the diet, there is a steady decrease in the loss of non-DNA cellular material as indicated by DFFT and decreasing DNA/mg DFFT ratio.

It has been suggested that observed variability in normal and experimental mammary gland growth may be due, in part, to sub-optimal endogenous secretion rate of hormones which stimulate mammary gland growth. Griffith and Turner[4] reported the influence of thyroxine upon mammary gland growth of pregnant rats and has shown increased mean DNA by 22%. Nelson[8] compared the effect of different levels of dietary casein on the lactational performance of rats as judged by the number of young weaned and weight changes in the dam and pups. Pregnancy was maintained in the absence of dietary protein in all animals injected with both estrone and progesterone[9]. In adult rats, the absence of dietary protein resulted in a moderate increase in the hypophyseal content of FSH and ICSH, whereas the anterior lobes of young rats were markedly depleted of both hormones[10]. An increase in dietary protein resulted in increased milk yield but with no significant changes in percent protein in the milk[11]. Several experimental observations indicate that the thyroid hormones can influence prolactin secretion. The galactopoietic action[12], lobuloalveolar develpoment of mammary gland[13], may be partially the result of synergistic effect between prolactin and thyroxine. Recently we[3] have reported that protein-free and 5% protein diet caused decreased TSR and food consumption. It is consistent with the finding of decreased mammary gland DNA at the same level. The data obtained in the present experiment also suggests that 10–20% protein in the diet is able to maintain body growth.

Zusammenfassung. Nachweis, dass der gesamte DNS-Gehalt in der Brustdrüse bei Tieren mit proteinfreier oder 5%iger Proteinnahrung im Vergleich zu der Kontrollgruppe herabgesetzt ist. Wird Protein in der Nahrung um 10–20% erhöht, so bleiben DNS-Gehalt und Körperwachstum konstant.

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