Influence of Gentling on Physiology of the Rat. (22420)

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It appears that various physiological processes in the albino rat can be altered by handling or gentling the animal for a prescribed time each day. It has been shown that gentled rats survive certain surgical procedures better than non-gentled animals(1-3) and that gentled animals possess greater discrimination learning capacity(4). Both the Weininger group(5,6) and our group(7) have studied effects of gentling on growth, thyroid metabolism and ability to withstand certain forms of stress. Weininger found that rats grew better and survived prolonged starvation better than non-gentled controls. We also found a relationship between gentling and growth, and attempted to enhance differences between gentled and non-gentled rats by subjecting animals to stress of starvation or electrical shock.

Methods. Sprague-Dawley weanling male rats weighing 40-44 g were distributed at random into groups of 8 to 12 rats each. All animals were housed in individual galvanized iron cages having wire mesh bottoms, and were fed commercial rat food* and water *ad libitum*. Weekly records were kept of individual weight gains and food and water consumptions. All animals from the gentled groups were removed from cages for 10 min. each day and were fondled individually by an investigator. At the conclusion of each experiment, all animals were injected intraperitoneally with 1 ml of I-131 carrier-free iodide (50 μc), and the animals sacrificed and autopsied 24 hr later. The carcasses were suspended by the tail, and measurements of length were made from tip of nose to anus. Thyroid glands were removed *in toto* and digested with NaOH until completely hydrolyzed. Aliquots were counted in a Nancy Wood Scintillation Well Counter to standard deviation of 2%, and percent dose calculations were made by comparing these counting rates with a 1:1000 dilution of standard dose. The shocking device consisted of induction coil connected to copper wire grid threaded into bottom of small cardboard box. The animal was held securely by tight fitting lid, and repeated short electrical shocks were administered for 10 min. Entire carcasses (except tails) of all animals in Exp. 1 and 2 were ground to homogenous mixture in meat grinder. Samples were analysed for total moisture, neutral fat and Kjeldahl nitrogen. Arithmetic means, standard deviations and errors were calculated, and a Student *t* test was made of significance of differences. A *p* value of .02 or less was considered to be significant.

Results. Twelve rats in each group were employed in first 2 experiments, and 12 and 9 rats respectively in single and grouped tests of Exp. 3. A summary of data is given in Table I. Gentled animals, whether individually caged or grouped, showed consistently and significantly better growth and food utilization values than non-gentled animals. Thyroid I-131 uptake measurements were always lower for gentled animals, although the significance of these differences was questionable. On the other hand, neither gross pathological disturbances nor differences in moisture, fat and nitrogen values for gentled and non-gentled animal carcasses could be found.

An experiment was carried out with 4 groups of 12 animals each, fed food and water *ad libitum* for 1 wk. after weaning. Two of these groups were gentled in the usual manner, the remaining 2 were not. At the end of this time, one gentled and one non-gentled group was placed on moderately restricted food intake of 8 g/day for first wk. and 10 g/day thereafter. The other gentled and non-gentled groups were alternately subjected to complete starvation for 48 hr and recovery. Usual growth and food utilization differences between gentled and non-gentled animals were evident for the first wk., but

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TABLE I. Summary of Physiological Differences Observed between Gentled and Non-Gentled Animals.

	Test No.	G-SC	NG-SC	G-DC	NG-DC	G-SC *vs* NG-SC	G-DC *vs* NG-DC
		Groups — Arithmetic means				Compare — p values	
Wt gain (g)	1	187.2	169.8			.007	
	2	189.9	154.3	163.0	145.5	<.001	.026
	3	155.7	145.1	153.6	143.7	.04	.05
Food utilization (g)	1	3.62	3.98			.008	
	2	3.28	3.65	3.40	3.76	<.001	.002
	3	3.31	3.64	3.40	3.63	"	.08
Carcass length (cm)	1	19.99	18.90			<.001	
	2	19.48	18.80	19.68	18.51	"	<.001
	3	20.27	19.56	20.30	19.55	"	"
Thyroid uptake (% dose)	1	9.40	9.65			.75	
	2	2.60	3.80	2.99	3.22	.005	.3
	3	2.80	3.07	2.67	2.72	.03	.8

G and NG designate gentled and non-gentled animals. SC and DC designate single caged and double caged animals.

these were essentially irradicated during food restriction or starvation (Table II, Fig. 1).

A further experiment was carried out in which animals were gentled for 2 wks. prior to stress. Then 8 rats from each group of gentled and non-gentled animals were sacrificed and carcass lengths and thyroid uptakes measured. Remaining animals were divided into the following groups of 8 rats each: gentled-shocked, non-gentled-shocked, gentled-starved, non-gentled-starved and non-gentled controls. Growth and food utilization were measured for the next 2-3 wks., then animals were sacrificed, autopsied and carcass length and thyroid uptakes were measured. Data are summarized in Table III and Fig. 2 and 3. With respect to starved animals, there were consistent differences between weight gains, thyroid uptake values and carcass lengths of gentled and non-gentled groups. Non-gentled-starved animals were unquestionably inferior to non-gentled controls in all categories. In shocked groups, gentled animals lost their initial advantage, and eventually attained similar weight gains as non-gentled group (Fig. 3). Therefore, weight gains and food utilization figures for gentled animals during the second 2 wk. period were relatively inferior.

Discussion. The first 3 experiments demonstrate that gentled animals grow better, utilize food more efficiently for weight gain, and tend to exhibit lower thyroid I-131 uptake measurements than non-gentled controls. Although the thyroid differences are too small to be statistically significant, they are consistently in the same direction and suggest that gentling may lower basal metabolism of

TABLE II. Summary of Physiological Differences Observed between Gentled and Non-Gentled Animals Subjected to Food Restriction or Electrical Shock for 3 Weeks.

Measurement (at 4 wk)	G-R	NG-R	G-St	NG-St	G-R *vs* NG-R	G-St *vs* NG-St
	Groups — Arithmetic means				Compare — p values	
Wt gain (g)	90.4	96.1	77.8	76.0	.08	.78
Food utilization (g)	3.32	3.16			.17	
Carcass length (cm)	17.90	18.31	18.1	17.80	.01	.07
Thyroid uptake $\bar{c}$ food (% dose)	27.9	22.17	12.0	17.2	.01	.02
$\bar{s}$ food			25.9	25.0		.74

G and NG designate gentled and non-gentled animals. R and St designate restricted food intake and starved animals.

TABLE III. Comparison of Growth, Food Utilization, Carcass Length, and Thyroid Uptake Values of Gentled and Non-Gentled Animals under Stress of Starvation or Shock.

	Second 2 weeks			Second 3 weeks			Compare			
	G-Sh	NG-Sh	NG-C	G-St	NG-St	NG-C	G-Sh *vs* NG-Sh	NG-Sh *vs* NG-C	G-St *vs* NG-St	NG-St *vs* NG-C
	Arithmetic means						p values			
Wt gain (g)	50.0	63.6	85.2	55.0	47.3	113.2	.005	<.001	.05	<.001
Food utilization (g)	5.6	4.3	3.32			3.36	.001	"		
Carcass length (cm)	18.97	19.28		18.72	17.78	20.17	.01		.004	"
Thyroid uptake (% dose)	24.1	19.9		14.28	17.53	12.03	.13		.07	.02

G and NG designate gentled and non-gentled animals. Sh, St, and C designate shocked, starved and control animals respectively.

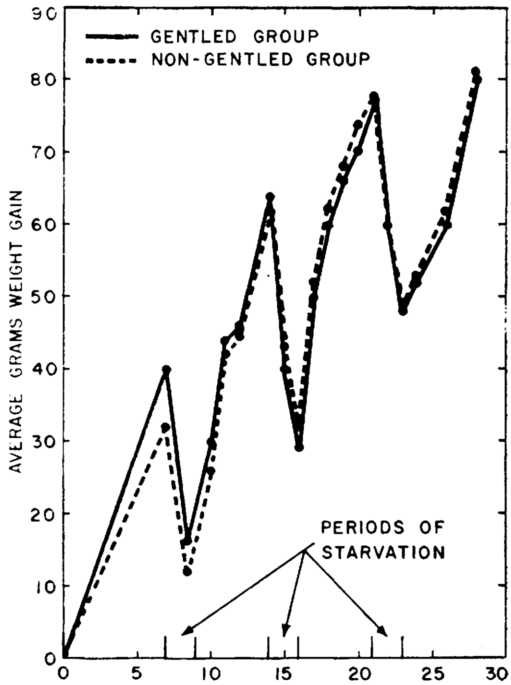

FIG. 1. Effect of pre-gentling for 1 wk on growth of animals subjected to starvation.

the rat by influencing the amount of thyroid hormone produced. This might be envisioned as alteration in amount of stimulation received by the hypophysis from the hypothalamus, which might in turn alter the amount of thy-

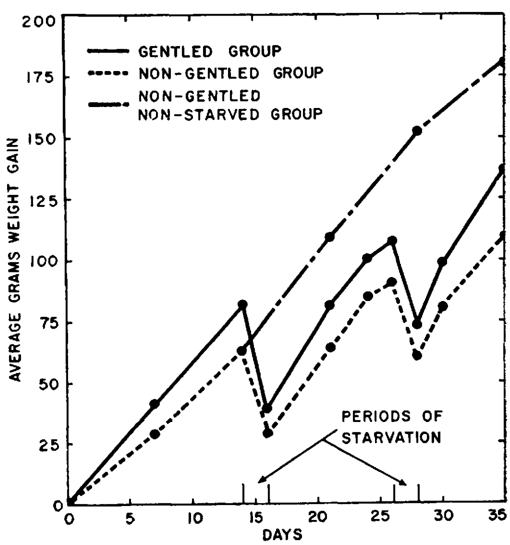

FIG. 2. Effect of pre-gentling for 2 wk on growth of animals subjected to starvation.

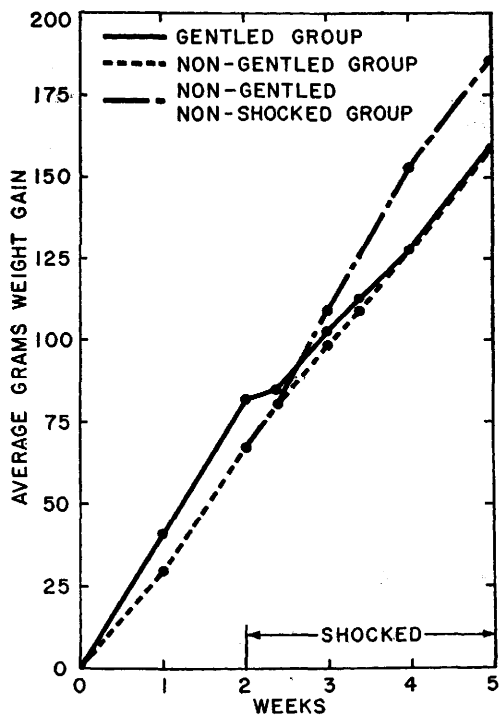

FIG. 3. Effect of pre-gentling for 2 wk on growth of rats subjected to electrical shock.

roid stimulating hormone produced by the hypophysis. The importance of the hypothalamic-hypophyseal stalk in eliciting a thyroid response to cold stress is well known(8).

It was observed in stress experiments that gentling failed to alter the effects of starvation or food intake restriction, provided that animals were given only 1 wk. of gentling before initiation of stress (Table II, Fig. 1). When the pre-stress gentling period was extended to 2 wks., gentling appeared to give starved rats an advantage which was extended over the entire period of stress (Table III, Fig. 2). In Weininger's experiments, animals were gentled for 3 wks. prior to prolonged starvation period. All non-gentled rats succumbed whereas none of the 6 gentled animals died as a result of this experience. Therefore it appears that 2 or more wks. of pre-stress gentling are necessary to influence the animal's ability to withstand the stress of starvation.

In any given experiment, thyroid I-131 uptake values for starved animals were higher than those for non-starved controls (Table III). Animals which were without food when uptake measurements were made, had higher values than those placed back on food. These observations coincide with those of Catz *et al.* (9) who have shown that I-131 uptakes performed after starvation are much higher because the animal received no iodide during this period, and not because of any obvious structural changes in thyroid gland. Therefore thyroid uptake measurement may not be valid for ascertaining the status of thyroid activity in starved animals. However, non-gentled animals in all cases showed higher uptake values than gentled groups, and if these differences are real, non-gentled animals with higher metabolic rates would naturally suffer greatest weight loss under stress of starvation.

When electrical shock was employed, gentled animals lost the growth and food utilization advantage acquired during the first 2 wks. of gentling (Table III, Fig. 3). Shocked animals in both groups had markedly higher uptake values than non-gentled-non-shocked controls, and gentled-shocked animals had higher uptake values than non-gentled-shocked animals. Although these latter differences were not statistically significant, the overall change in thyroid uptake suggests an increase in thyroid function for the shocked groups. This in turn might account for the inferior growth and food utilization performance of these animals.

Summary. Small but statistically significant differences in growth and food utilization were produced in the albino rat through gentling. Gentled animals also showed a trend toward a lowering of thyroid activity. When animals were gentled for 1 wk. preceding stress, no significant differences in growth, food utilization, carcass length or thyroid function could be found between gentled and non-gentled animals. If this initial gentling period was extended to 2 wk., gentled-starved animals extended their growth and food utilization advantage over the non-gentled group, and showed a consistent trend toward lower thyroid function. Animals which were gentled for 2 wks. preceding electrical shock, lost their growth and food utilization advantage over the non-gentled group,

and both groups were markedly inferior to the non-shocked controls. Thyroid uptake measurements indicated that the basal metabolism of electrically shocked rats was increased over that of non-shocked animals, but no statistically significant differences existed between gentled and non-gentled groups.

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Circulating Rat Cells in Lethally Irradiated Mice Protected with Rat Bone Marrow.* (22421)

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Murphy(1) established that (a) embryos lacking ability to resist growth of heterologous tissue could be rendered as refractory as adults by introduction of an isologous adult spleen or bone marrow and (b) adults, capable of resisting growth of heterologous tissue, could be rendered as receptive as embryos by pretreatment with sublethal total-body X irradiation. Rapidly growing tumor tissues were used as donor material. Over 30 years later, Owen(2) demonstrated circulating erythrocytes genetically and phenotypically distinct from the animal's own, which arose from embryonic erythrocytopoietic transplants of the co-twin through placental anastomosis. Successful homologous and heterologous tissue and protein acceptance by embryos and X-irradiated adults followed by a prolonged or even lasting period of tolerance to these agents has been reported(3-8). Lindsley *et al.*(9) demonstrated erythrocyte mosaicism in sublethally X-irradiated rats that were subsequently injected with homologous rat bone marrow.

Previous work(10) in this laboratory showed that C_3H x $101F_1$ mice receiving 950 r of total-body X radiation protected against radiation death by a single intravenous injection of an isologous bone marrow suspension responded relatively well to either rat or sheep RBC antigens 30 days later. However, lethally X-irradiated mice protected by an injection of heterologous bone marrow (Sprague-Dawley rats) failed to respond to rat RBC antigen and responded only very weakly to sheep RBC antigen 30 days later. The establishment of the degree of transplantation of rat hematopoietic tissue became necessarily important in an attempt to interpret the immune response of these heterologously protected mice.

Materials and methods. Normal 12-week-old C_3H x $101F_1$ mice, equally divided in sex, received 950 r and were subsequently protected with heterologous bone marrow suspension. Three types of control mice were run simultaneously: (a) nonirradiated normal, (b) irradiated (950 r) and protected with isologous bone marrow suspension, and (c) nonirradiated but injected with heterologous bone marrow suspension. The irradia-

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