Enhancement of Tumor Development in Physically-Stressed Mice Inoculated with an Oncogenic Virus

In the course of experiments where mice were subjected to a procedure of partial body-casting, it was observed that such treatment resulted in a decrease in the size of the thymus glands and spleen and a moderate increase in the size of the adrenal glands. The mice fit a description of animals subjected to a moderate stress[1,2]. The systemic actions of some adrenal steroids liberated during a phase of stress reactions are known to inhibit some cell-mediated immune reactions[3]. In particular, mice have been rendered immunologically incompetent toward oncogenic viruses including the Moloney Sarcoma virus, as a result of cortisone administration[4-6]. It was of interest, therefore, to examine the influence of partial body-casting on the susceptibility of immunologically competent mice to an oncogenic virus, since the casting procedure appeared to induce a systemic stress reaction.

Materials and methods. The mice used in the experiment were 8-week-old males of the CBA/J strain which had been acclimated to their housing for 1 week prior to the onset of the experiment. The mice were housed 4 per cage to avoid a possible stress effect of crowding. There were 2 cages for each experimental group. 8 groups of mice were established. 4 of the groups (experimentals) were subjected to partial bodycasting and remained casted during the entire experiment. The casts were snug-fitting circumferential plaster of Paris casts covering the chest and upper abdomen of the mice[7]. 3 days after the experimental mice were casted, all animals, experimental and control (not casted), were inoculated with murine sarcoma virus (MOLONEY, Lot No. 206), which was a gift to one of us (M.Z.) from Dr. J. B. MOLONEY of the National Cancer Institute, National Institutes of Health, Bethesda, Maryland. 4 concentrations of the virus were used, 1×10^{-1}, 1×10^{-2}, 1×10^{-3}, and 1×10^{-4}, based upon dilution of the original material with a phosphate-saline buffer[8]. All animals were inoculated with 0.1 ml i.m. as described by BLUMENSHEIN and MOLONEY and the animals were evaluated daily by the criteria used by these workers[9].

To study the influence of carrying the cast on the thymus glands of the mice both histologic and chemical analyses were performed. For the histologic examination, 5 mice of the same breed, sex and prior history as those used in experiment 1 were subjected to partial body casting and were housed in one cage. A second group of 5 mice was subjected to the same degree of anesthesia and handling as were the experimental mice. After 3 days all of the mice were killed and their thymus glands were placed in 10% buffered formalin. Hematoxylin-eosin stained sections were then prepared.

The chemical composition of the thymus gland was determined on each of ten mice (stressed for 3 days) and on each of 10 control mice. The glands from each mouse were homogenized in 2 ml of 0.9% NaCl. 0.5 ml of the homogenate was then analyzed for protein by the method of LOWRY et al.[10]. A 1 ml aliquot was then treated with 2.5 ml of cold 10% trichloroacetic acid (TCA). The mixture was shaken and centrifuged. The supernatant fluid was discarded and the precipitate was washed with two 2.5 ml portions of cold TCA. The precipitate was mixed with 5 ml of 5% TCA and the mixture was heated for 30 min in a boiling water bath. The solution was cooled and centrifuged. 1 ml aliquots were used for the analysis of DNA by the method of BURTON[11] and for the analysis of RNA by the method of MEJBAUM[12].

Results and discussion. The data (Table I) demonstrate that mice wearing the cast have a higher incidence of tumor than do control mice. This was true for animals in

Table I. Incidence of tumor

	No. of mice	Inoculum	Days Post Inoculation				
			5	7	11	23	30
Control	8	1×10^{-1}	0	3	8		
Casted	8		2	8			
Control	8	1×10^{-2}	0	0	1	4	4
Casted	8		0	0	4	8	
Control	8	1×10^{-3}	0	0	2	4	4
Casted	8		0	0	3	7	7
Control	8	1×10^{-4}	0	0	0	0	0
Casted	8		0	0	0	2	3

The values shown are the number of animals in each group which had shown palpable tumors by the stated post-inoculation date. Tumors appeared earliest in mice receiving the highest concentration of virus and subjected to stress.

Table II. Mean tumor score at various times after initial detection of tumor

	Inoculum	Days after initial detection of tumor		
		3	5	10
Control	1×10^{-1}	1.7	0.8	0
Casted		2.6	3.6	2.0
Control	1×10^{-2}	1.5	1.5	0
Casted		2.0	2.3	1.9
Control	1×10^{-3}	1.1	0.8	0
Casted		2.0	1.0	0.4
Control	1×10^{-4}	No tumor developed during this period		
Casted		1.2	0.8	0.5

The maximum score of casted animals is greater than that of control mice. In casted mice the tumor is growing at a time when regression is occurring in control animals. The tumors were graded on a scale of 1–4 as used by BLUMENSHEIN and MOLONEY[9].

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Table III. Regression of tumors

	Inoculum	No. of mice	No. of mice with tumors	No. of tumors regressed in 30 days	Time required for regression of tumors (days)	No. of mice with tumors after 30 days
Control	1×10^{-1}	8	8	8	6	0
Casted		8	8	4	10	4
Control	1×10^{-2}	8	4	3	10	1
Casted		8	8	5	11	3
Control	1×10^{-3}	8	4	3	5	1
Casted	8	8	7	6	9	1
Control	1×10^{-4}	8	0	0	–	–
Casted		8	4	4	11	0
Totals:						
Control		32	16	14	6	2
Casted		32	27	19	10	8

Tumors failed to regress in stressed animals as rapidly as in non-stressed mice.

all groups except those receiving the most concentrated (1×10^{-1}) inoculum. In these animals the frequency of the tumor in control mice was 100%. The effect of casting in increasing tumor incidence is most clearly seen in animals receiving the lowest dosage of virus.

At a 1×10^{-4} dose of inoculum, none of the control mice developed tumors, while 3 of 8 of the casted mice did. At 1×10^{-3} dosage of virus, 4/8 control mice developed tumors as compared to 7/8 in the experimental group. At 1×10^{-2} dosage, 4/8 controls developed tumors compared to 8/8 in the group subjected to casting. These results are similar to those seen in mice inoculated with MSV and subjected to immunosuppression by cortisone[6] or anti-lymphocytic serum[8].

The latency period (time required for the development of a palpable tumor) was dose-dependent in both the experimental and control groups; for any given concentration of viral inoculum, the latency period in casted animals was decreased compared to the controls. Thus by day 7, all casted animals receiving the highest viral

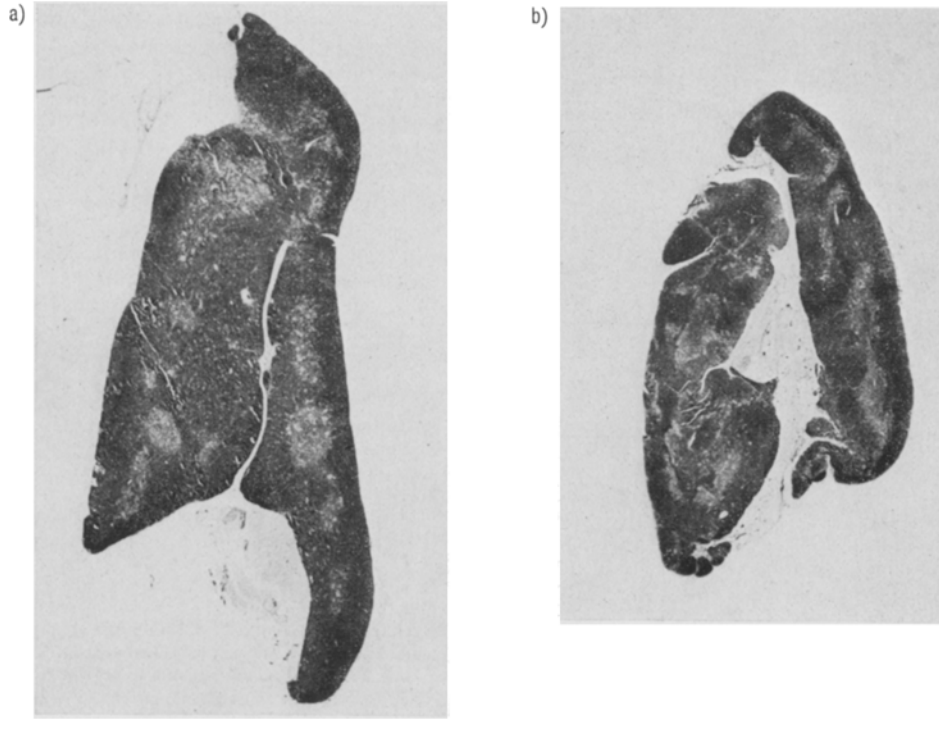

Fig. 1. a) Low power magnification of the thymus gland of a normal mouse stained with hematoxylineosin. Cortical areas are darkly stained. The medulla is composed of discrete zones of lightly stained areas. b) Identical magnification of a section of the thymus gland from a mouse stressed by casting for 3 days. The glands are obviously smaller and there is a distortion of the normal architectural pattern. The medulla appears diffuse due to the loss of cortical zones between the medullary patches.

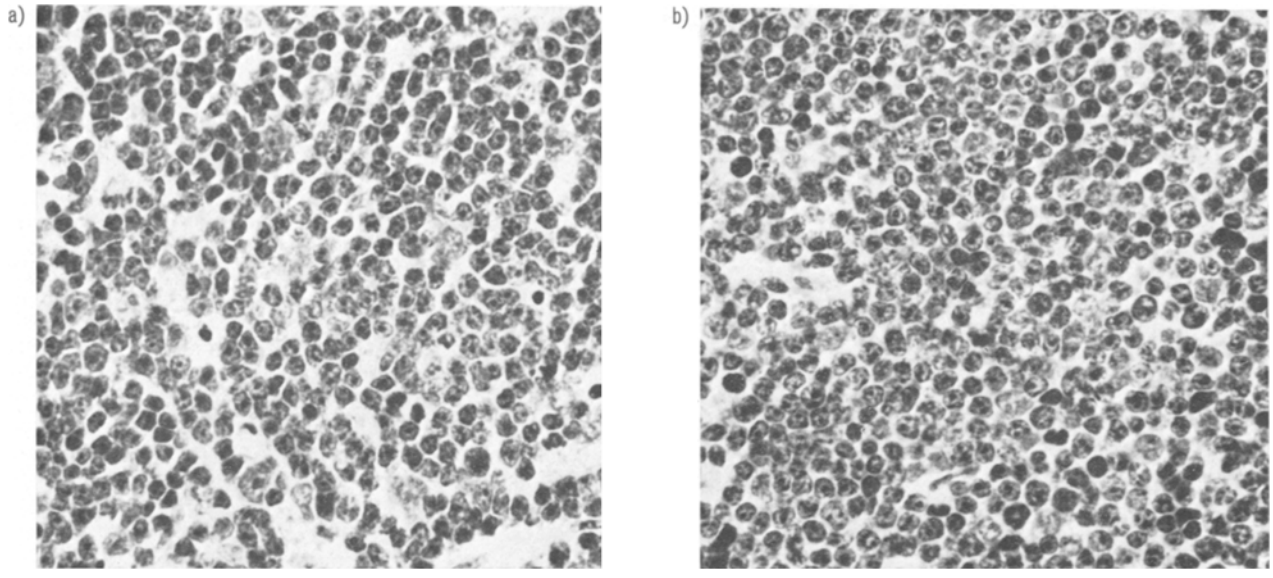

Fig. 2. a) High power magnification of a thymus section from a normal thymus gland showing the normal architectural pattern of cortex of the thymus. b) Similar magnification of the thymus from a stressed mouse. The architecture is distorted by the presence of numerous histiocytes.

inoculum had developed tumors while 3/8 of the controls had tumors. None of those receiving lower viral inocula had tumors at that time.

The maximum tumor size develped (score) was greater in the casted mice than in the control animals (Table II). The maximum tumor size (3.6) was found in mice inoculated with the highest concentration of virus and subjected to casting. This score was twice that of the control noncasted mice. Similarly at viral doses of 1×10^{-3} tumor score of the casted mice was 2.0 compared to 1.1 for the control group. Although the maximal tumor score was smaller in control mice as compared to the casted mice, it was achieved rapidly, once a detectable tumor had appeared.

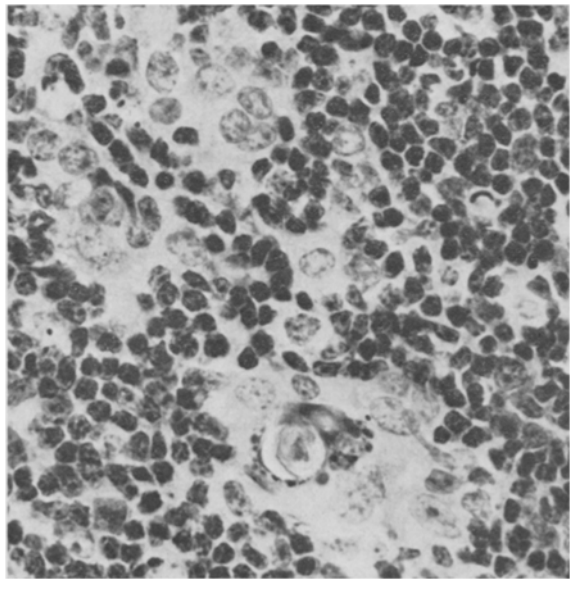

Fig. 3. Section of a thymus gland from a stressed mouse showing the margin between the medulla and cortical region. The area in the lower center is composed of aggregates of epithelioid elements concentrically arranged about what appears to be a Hassal body.

Tumor regression was more rapid and complete in control mice as conpared to casted mice (Table III). Regression was poorest in casted mice inoculated with the highest concentrations of the virus. Thus 7/15 of these mice were tumor bearing after 30 days, while 1/12 of the controls receiving the same inocula had tumors at that time. Tumor regression is common in mice of this age and strain and failure to obtain regression in casted mice may be considered to result from an immunodepression associated with stress. Repeat experiments using more mice of the same age, strain and sex have yielded similar results, indicating that the experiment is reproducible.

The influence of stress on the course of tumor development was interpreted as being related to thymic involution, associated with stress. We have examined the influence of partial body-casting as a stress on the chemical composition of the mouse thymus glands in an effort to determine if stress results in a preferential loss of cells or protein of these glands. CBA/J mice similar to those used in the tumor studies were divided into 2 groups. 1 group was subjected to casting, the other served as a control group. After 3 days the mice were killed and their thymus glands were analyzed (Table IV) and thymus tissue was taken for histologic examination.

The data show that with the stress used the thymus weight decreased 44%. This was due mainly to the loss of cortical cells as shown by a 51% loss in DNA content of the gland and a 52% loss in its RNA. Protein loss was 40% indicating either a lesser loss of thymic secreted proteins such as thymosin or replacement of some cortical cells by edema fluid. The magnitude of the changes in thymic composition induced by the stress of partial body casting are similar to those that followed the administration of 15 μg cortisone acetate daily for 3 days.

The microscopic examination revealed that the normal architecture of the thymus gland was altered in stressed mice. There appeared to be a smaller number of small lymphocytes due to stress and a proliferation of epithelioid elements. Hassal bodies were also involved in agreement with earlier findings of SELYE[13]. If stress or cortisone

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treatment result in impaired function of these structures, then the endocrine function of the thymus gland as well as its role in providing circulating cells would be disturbed. The hormonal function of the thymus gland has been described by WHITE and GOLDSTEIN[14]. The thymic hormone, thymosin, is thought to be produced in the medulla, probably by the Hassal bodies.

The experiements provide a way of measuring the influence of a physical stress on the course of a viral disease. The stress is not an unusual one, nor would one have considered it to be especially severe. Since the stress is presistent and uniform with respect to duration and may be uniformly applied to animals, it offers a model for studying agents which may enhance the immune responses in stressed animals. Such agents may be useful in the treatment of acute or quiescent stages of viral diseases.

Table IV. Effect of stress on thymus

Groups	Body weight (g)	Thymus			
		Weight (mg)	DNA (μg)	RNA (μg)	Total Protein (mg)
Stressed	18.0	20.4	450	140	3.4
Control	18.0	36.6	914	291	5.7
P value	1.0	0.001	0.001	0.001	0.01

There were 10 mice per group. *P* value was calculated using the *t*-test. Stress produced a sharp and similar decrease in the DNA and RNA content of the thymus glands.

Riassunto. L'applicazione di un parziale bendaggio nei topi produce uno stato di fisiologica tensione caratterizzata, in parte, dall'involuzione della ghiandola del timo. Topi sottoposti a una tale tensione sono più suscettibili allo sviluppo dei tumori quando vengono inoculati con un oncogenico virus.

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[15] We are appreciative of the help provided by Dr. K. NAKAO and Dr. B. BENNETT of the Department of Pathology, Albert Einstein College of Medicine, for interpreting the microscopic sections. Supported by NIH grants No. CA 12383 and No. 5K5-GM-14, 208. This work is dedicated to Dr. DAVID STATE.

Uptake of Dopamine by the Neural Lobe of the Pituitary Gland During Postnatal Development of the Rat

A relatively high monoamine oxidase (MAO) activity has been observed in the neural lobe of the pituitary gland of the rat, both in the neurosecretory axons and in the glial cells (pituicytes): so far the functional significance of this observation is conjectural[1]. Except for the noradrenaline containing sympathetic fibres innervating the blood vessels, the only monoamine-containing structures in the neural lobe are 1. the nerve plexus belonging to the tuberohypophyseal system, which contains mainly dopamine[2,3] and 2. a few mast cells containing serotonin[4]. One possible function of MAO would be inactivation of monoamines liberated from the nerves. Another possibility is that MAO would be needed to inactivate monoamines liberated from the median eminence, from which minor part of the portal vessels enters into the neural lobe[5]. In the portal blood, however, only minutely detectable amounts of monoamines have been found[6].

MAO activity increases in the brain tissue 8-fold from the first postnatal day to the adult age[7]. We have followed the uptake of dopamine into the neural lobe during this postnatal period.

Twenty-five female rats aged 1, 7, 14, 34 days and over 2 months old rats (5 in each age group) were injected i.p. with dopamine chloride (Orion, Helsinki), 100 mg/kg body weight, $^1/_2$ h before decapitation. The pituitary glands were processed for formaldehyde-induced fluorescence (FIF) according to the method described by ERÄNKÖ[8], and embedded in Epon resin; 2–5 μm thick sections were viewed and photographed with Leitz Ortholux fluorescence microscope fitted with an epi-illuminator[9] and with appropriate filter combinations. Subsequently the same or serial sections were stained with toluidine blue for light microscopy to identify the fluorescent structures. Some unstained sections were also examined with dark-field microscopy.

After the dopamine injection, a strong, green FIF developed in the neural lobe of the younger groups of the rats. The strongest fluorescence was observed in the neural lobes of 1-day-old rats. The intensity of the FIF decreased evenly with the age of the rats. At 2 months of age, the dopamine injection caused only a weak, just detectable fluorescence.

The FIF of young rats was distributed in the cytoplasm around the nuclei of the pituicytes, in the neurosecretory axons and in the processes of the pituicytes, where it was found to be granular. The intensity of the FIF faded only slowly. During the development the number of the nuclei of the pituicytes per unit area decreased. No uptake was observed in the cells of the pars intermedia and the pars distalis, as also reported by DAHSTRÖM and FUXE (1966)[10], when only dopamine was given. According

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