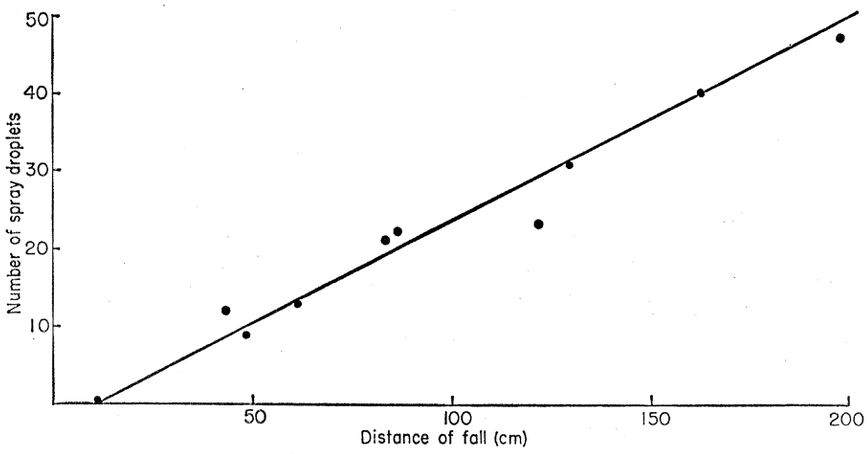

Fig. 3. Number of spray droplets as a function of the distance that a water drop 3 mm in diameter falls.

electrodes during the formation of the spray droplets, a grounded plate was placed between the water and the electrodes. The ratio of the charge q on a droplet to its mass m was determined from the formula

$$q/m = (g/E) \tan \theta$$

where θ is the angle which the path of the droplet makes with the vertical direction and g is the acceleration due to gravity. The angle θ was determined by photographing the path of a droplet in stroboscopic illumination as it fell through the electric field.

The direction in which the spray droplets were deflected in the applied field showed that nearly all of them carried a negative charge. The measured values of q/m varied from about 4 to 28 electrostatic units per gram. From estimates of the size of the droplets from the photographs, approximate values were obtained for the magnitudes of the charges on the spray droplets. The results showed that the larger droplets had the greater charge. For example, droplets 125, 200, and 400 μ in diameter had charges of 9.2×10^{-6}, 5.4×10^{-5}, and 9.4×10^{-4} electrostatic units, respectively. The distance that the drop fell, on the other hand, appeared to have little effect on the charges carried by the spray droplets over the range investigated.

P. V. HOBBS
A. J. KEZWEENY
Department of Atmospheric Sciences, University of Washington, Seattle

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Loss of Thymus-Distinctive Serological Characteristics in Mice under Certain Conditions

Abstract. *The serological properties of thymus cells of inbred mice bearing Ehrlich ascites tumors and treated with cortisol were investigated. Thymus-distinctive antigenicity and sensitivity to the cytotoxic effect of guinea pig serum and rabbit serum were significantly decreased, without change in reactivity to H-2 isoantibodies.*

Thymus cells of mice show several distinctive serological characteristics. The thymus cells of all mouse strains tested are sensitive to the cytotoxic effect of guinea pig serum (*1*); cells of some strains, such as SJL/J and A, possess a thymus-distinctive (TL) antigen that is absent from all other lymphoid cells (*2*). Normal thymus cells of other strains, such as C57BL, do not possess this antigen. Evidence has recently accumulated that the thymus may be constantly repopulated by stem cells from the bone marrow (*3*). Cells derived from the bone marrow or spleen of strain-A mice become TL-positive and sensitive to guinea pig serum upon repopulating the thymus of lethally irradiated recipients, of even TL-negative strains (*4*). It seems that stem cells entering the thymus may be constantly induced to acquire thymus-distinctive characteristics.

Since tumor growth (*5*) and administration of adrenocorticosteroid hormones (*6*) profoundly affect the thymus, their effect on the expression of thymus-distinctive characteristics was studied with inbred A, C57BL/6, and SJL/J mice. Pools of guinea pig and rabbit serums were obtained from random-bred animals. The isoantiserum used to detect the TL antigen was prepared by repeated intraperitoneal administration of thymus cells of the A strain to (BALB/c × C3H) F_1 hybrids (*2*). The isoantiserums used to detect H-2 isoantigens of the A and C57BL/6 strains were obtained following repeated intraperitoneal administration of spleen cells of the A and C57BL/6 strains to the reciprocal strains. The reaction of the isoantibodies with spleen or thymus cells was studied by a modification (*7*) of the cytotoxic test of Gorer and O'Gorman (*8*). The guinea pig serum used as source of complement was absorbed with murine tissues to remove its cytotoxicity to mouse thymus cells before being employed in the cytotoxic tests. The sensitivity of thymus cells to the cytotoxic effects of guinea pig and rabbit serums was tested by adding 25,000 thymus cells, suspended in 0.025 ml of normal saline, to 0.025 ml of serially diluted, unabsorbed, guinea pig or rabbit serum. A volume of 0.04 ml of cortisol acetate suspension (*9*) was administered subcutaneously. Fifty million Ehrlich ascites tumor cells were administered intraperitoneally. All the mice were maintained on regular Purina diet and water ad libitum; they were killed at various intervals after treatment, and the thymus and spleen cells were tested.

Within 18 hours of subcutaneous administration of 1 mg of cortisol acetate, the reactivity of A and SJL/J thymus cells with TL antibody disappeared completely (Tables 1 and 2). No TL antigenicity could be detected in the thymus cells within 6 days of administration of cortisol; on the 7th day some sensitivity to the TL antibody reappeared, and 1 day later the reactivity of the thymus cells returned to normal or became even slightly higher. The sensitivity of the thymus cells to

guinea pig serum was lost at the same time as the reactivity with TL antibody. The thymus cells were completely refractory to guinea pig serum until the 7th day after administration of cortisol, but regained full sensitivity 1 day later. Normal rabbit serum is cytotoxic not only to mouse thymus cells but also, to a lesser degree, to mouse bone marrow cells (*10*). In cortisol-treated mice of strain A, the sensitivity to rabbit serum was completely lost in parallel with the loss of sensitivity to guinea pig serum. On the other hand in SJL/J mice the sensitivity to rabbit serum was only slightly reduced and, apart from one animal, did not disappear completely.

No serological changes were detectable in the thymus cells within 2 days of intraperitoneal transfer of the Ehrlich ascites tumor (Tables 1 and 2); but onward from the 4th day after implantation the thymus cells of both A and SJL/J strains completely lost their sensitivity to TL antibody and to guinea pig serum. The cells failed to regain sensitivity after prolonged growth of the tumor. Rabbit serum had a variable effect, but it retained some cytotoxic effect on the thymus cells of most tumor-bearing mice.

No decrease in sensitivity of the thymus cells to H-2 antibodies was detectable in the experimental animals; if anything, the cells of tumor-bearing or cortisol-treated mice showed higher sensitivity to H-2 antibodies than did normal thymus cells. Similarly, no significant changes in H-2 isoantigenicity were observed in the spleen cells.

Thymus cells of cortisol-treated or tumor-bearing mice that were resistant to TL antibody in the cytotoxic tests were tested for capacity to absorb TL antibody; they were incapable. The growth of Ehrlich ascites tumor and administration of cortisol lead to disappearance of sensitivity to guinea pig serum of thymus cells in mice of several other strains (C3H, C57BL/6, RIII).

Our study showed that the thymus cells of mice treated with cortisol or bearing Ehrlich ascites tumor lose their thymus-distinctive properties. Both administration of corticosteroid hormones and growth of tumors lead to thymic involution (*5*, *6*). While tumor growth may cause increased secretion of adrenal corticosteroid hormones (*11*), the thymic involution in tumor-bearing animals seems to be independent of the pituitary-adrenal axis (*12*).

Corticosteroid hormones have been shown to stabilize cell and lysosomal membranes in vitro (*13*). Large doses of cortisol may inhibit the lysis in vitro of red blood cells by antibodies and complement (*14*). However, the resistance of thymus cells to lysis by guinea pig serum and by TL antibody, observed by us, cannot be explained by stabilization of cell membranes; such a nonspecific stabilization should have equally affected the sensitivity of the thymus cells to H-2 antibodies. Moreover, absorption experiments confirmed the disappearance of the TL antigenicity from the cells.

Table 1. Effects of tumor growth and administration of cortisol on serological properties of thymus cells of strain SJL/J mice. Titer defined as the highest dilution of serum that showed deformation and staining of 50 percent or more of the test cells; each value is for a different mouse.

Duration (days)	Cytotoxic titer		
	Guinea pig serum	Rabbit serum	TL antibody
	No treatment		
	32	128	1024
	Ehrlich ascites tumor		
2	<2, 32	32, 64	512, <4
4	<2, <2	<4, <2	<16, <4
8	<2	8	<2
	Cortisol treatment		
1	<2	4	<4
2	<2, 8	8	<4, 64
4	<4	8	<4
6	<2	<2	<4
7	<2	8	128
8	32	32	2048
10	32	64	1024
12	32	64	1024

Allogeneic skin grafts obtained from tumor-bearing (*15*) or cortisol-treated animals (*16*) survive longer than allografts obtained from normal donors. No decrease in H-2 antigenicity of tissues of tumor-bearing or cortisol-treated mice could be demonstrated (*17*). It is, however, possible that, like the TL antigen disappearing from the thymus, antigens, other than H-2, may disappear from the organs of treated animals.

The following two hypotheses may explain the antigenic changes observed by us in the thymus:

1) Tumor growth or administration of cortisol may have a selective detrimental effect on thymus cells possessing thymus-distinctive serological properties, leaving in the thymus only a cell population devoid of such properties. This hypothesis seems to be supported by the cytotoxicity of corticosteroid hormones in vitro for thymus cells (*18*), and by the characteristic increase in population of large cells in the involuting thymus (*19*). One should stress, however, that the abrupt reappearance of thymus-distinctive serological properties on the 7th to 8th day after administration of cortisol must reflect a process occurring within the thymus, since no TL-positive and guinea pig serum–sensitive cells that could repopulate the thymus have been found outside the thymus.

2) The induction of thymus-distinc-

Table 2. Effects of tumor growth and administration of cortisol on serological properties of thymus and spleen cells of strain A mice. Titer defined as the highest dilution of serum that showed deformation and staining of 50 percent or more of the test cells; each value is for a different mouse.

Duration (days)	Cytotoxic titer				
	Spleen cells, H-2	Thymus cells			
		H-2	Guinea pig serum	Rabbit serum	TL antibody
		No treatment			
	256	512	64	256	1024
	2048	2048	64	256	1024
			32	64	1024
		Ehrlich ascites tumor			
2	512	256			2048
	2048	2048	64	128	2048
4	512	1024	<2	8	<2
	2048	2048	<2	64	<4
6	256	1024			<4
	2048	2048	<2	<2	<4
7	512	512	<2	<2	<4
10	512	512	<2	32	<4
	256	1024	<2	8	<4
		Cortisol treatment			
1			<2	<4	<4
2	1024	1024	<2	<4	<4
4	2048	2048	<2	<2	<4
7	2048	2048	<2	<4	128
8	2048	2048	32	32	2048
10	2048	2048	32	64	1024
12	2048	2048	32	64	2048

tive serological properties in the stem cells entering the thymus may be inhibited. Inhibition may be due to either loss of the inductive capacity of the involuting thymus, or refractoriness of the stem cells to the inducing capacity of the thymus. Boyse *et al.* (*20*) have recently observed that expression of the TL antigen could be suppressed in the thymus of neonatal and adult mice by antibodies against the TL antigen; evidence was presented that this phenomenon was not due merely to masking of the antigenic sites by blocking antibodies; experiments in vitro showed that treatment of TL-positive cells with actinomycin D abolishes their capacity to modulate to TL-negative cells. Thus it seems that treatment with TL antibody triggers an active metabolic process leading to loss of the TL antigen.

Recent evidence is that administration of cortisol may inhibit synthesis of purine nucleotide and protein in the thymus, and that it may impair the ability of thymic ribosomes to incorporate amino acids (*21*). The changes in TL antigenicity and in sensitivity to guinea pig serum, observed by us, were very abrupt and complete within 1 day or 2. Since the expression of TL antigenicity and guinea pig serum–sensitivity in the thymus seems to involve an inductive process, the abrupt "switch off" and "switch on" of thymus-distinctive properties may reflect an effect on the genetic regulatory mechanisms of thymus cells.

While the antigenic changes observed in the involuting thymus could reflect inhibition of inductive processes, it now seems entirely possible that they could be accounted for by selection of a cell population lacking thymus-distinctive serological properties. Further information is necessary to determine which of these two mechanisms causes the changes.

M. SCHLESINGER
V. K. GOLAKAI

Department of Experimental Medicine and Cancer Research, Hebrew University–Hadassah Medical School, Jerusalem, Israel

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Heme and Globin Synthesis Control: Observations in vivo in Beta Thalassemia

Abstract. *After administration of glycine-2-^{14}C to a patient with thalassemia, the specific activities of heme and globin of F hemoglobin were consistently higher than those of hemoglobin A. After reaching a maximum, the ratio of the specific activity of heme to that of globin remained constant within each hemoglobin. Explanations considered include dilution by preformed subunits, differential turnover of hemoglobins, and possibly more than one heme-synthesizing pool.*

The biosynthetic relation between heme and globin is a problem of current interest, and the heritable abnormalities of hemoglobin synthesis, such as the thalassemias, provide an opportunity to obtain insight into this relation. The thalassemia mutations are considered to interfere with the rate of synthesis of globin chains, and there is no evidence of structural abnormality in the hemoglobins (*1*). Our study is concerned with the relation between the heme and globin moieties of hemoglobins A and F.

A splenectomized patient with β-thalassemia intermedia, who was producing a mixture of hemoglobins, A (56 percent), F (44 percent), and A_2 (1.1 percent), was given a single dose of 150 μc of glycine-2-^{14}C intravenously for the purpose of studying the labeling of hemoglobins A and F in the circulating red cells and the labeling of various pyrrole compounds excreted in the urine and feces (*2*). Washed red cells were hemolyzed, and the stroma was removed by toluene treatment and centrifugation. The hemolyzates were stored at −20°C for a few days and were then dialyzed overnight against phosphate buffer, *p*H 6.0 (0.05*M* sodium phosphate; 0.05*M* NaCl; 0.005*M* NaCN). Hemoglobins A and F were separated on a carboxymethyl Sephadex C-50 column (*3*). The phosphate buffer was also used to equilibrate the column and to elute hemoglobin F. To elute hemoglobins A and A_2, phosphate buffer (*p*H 7.0, in 0.1*M* NaCl) was used. The separation of the fractions was checked by starch-gel electrophoresis. The small amount of A_2 hemoglobin was included with the A fraction. The hemoglobin solutions were concentrated by pressure dialysis, and heme and globin were prepared from each (*4*).

Measured samples of solutions prepared from crystalline hemin were placed on a planchet in a thin layer, and their ^{14}C activity was measured in a gas-flow counter. Some activity was evident at 24 hours after the pulse labeling (Fig. 1), at which time the activity of the heme from hemoglobin F was already greater than that of the heme from hemoglobin A. This difference persisted throughout the period of study, the ratio of specific activities of heme (F/A) ranging from 1.4 to 2.1 (mean 1.7). The ratio taken from the smoothed curves is highest at day 1, then falls steadily, but after 18 days remains at approximately 1.5.