Effect of Irradiation and Cortisol on Thymus Fraction.* (27880)

AILENE HERRANEN AND WILLA K. BRUNKHORST (Introduced by R. I. Dorfman)
Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

The question of a sensitive and non-sensitive cell population must be considered when the effect of any agent on a tissue is measured. It has been reported that there are 2 types of cells in thymus, lymphocytes which are radiosensitive and reticulum cells which are radioresistant(1). These cell types also differ in sensitivity to glucocorticoids(2). Eichel and Roth have discussed the problem of defining changes in enzyme activity in extracts from organs which undergo atrophy, with a corresponding nitrogen loss(3).

Two methods for differential extraction of thymus have been described(4). This report concerns the effect of ionizing radiation and cortisol treatment on the composition of the cell fractions using these methods. Gross involution of the thymus caused by these agents virtually eliminates small lymphocytes from the organ. A comparison of fractions from normal tissue and atrophied organs should indicate the cellular origin of the fraction or of major components in the fraction.

Methods. Male rabbits, 3 to 4 months old, were exposed to 650 r of radiation from a Cobalt 60 source and sacrificed 72 hours later. Animals treated with cortisol were given 5 mg subcutaneously twice daily for 3 days and sacrificed 72 hours after the initial injection.

Thymuses were removed and pooled into respective control and treated groups. The fractionation procedures described previously were applied(4). Briefly, in Method I the tissue was sequentially extracted by stirring slowly in water, followed by saline. The residual tissue was extracted with saline at high speed in the Waring Blendor. In Method II, the tissue was separated into nuclear and cytoplasmic fractions by a procedure based on the method of Allfrey *et al.*(5). The residual tissue was extracted twice with 0.15 M NaCl to obtain a saline extract and a final residue of non-extractable material. Nuclei were fractionated by stirring first in H_2O, then saline, followed by 2 homogenizations in 0.15 M saline. All extracts were centrifuged for 1 hour at 23,000 $\times$ *g* to obtain the corresponding supernatant and sediment fractions which were dialyzed and lyophilized to obtain dry weights. Ribonucleic acid determinations were carried out using the procedure of Schmidt and Tannhauser(6).

Results and discussion. Method I. Dry weights of the non-dialyzable material in each fraction obtained by Method I are shown in Tables I and II. The results are expressed as grams per 100 g wet weight of thymus and as mg per thymus. Because of the dramatic drop in number of lymphocytes and decrease in mass of the tissue, the data expressed as yield per thymus should reveal which fractions were obtained from lymphocytes.

When expressed as g/100 g of thymus, the dry weight of Fraction A is higher in the irradiated and cortisol treated animals. The composition of the fractions from control and treated animals is different as determined by electrophoretic analysis. A larger weight fraction of serum albumin in Fraction A from treated animals indicates that a higher percentage of this fraction is due to serum proteins as compared with Fraction A from untreated animals. The amount of Fractions B, C, and D decreased in tissue from treated animals. Tables I and II also illustrate the loss of ribonucleic acid from most fractions.

Extraction of thymus by this method does not provide fractions which clearly differentiate radiation and hormone sensitive or radiation and hormone resistant cells. If a fraction originated from resistant cells the yield per thymus should not change, whereas the yield per 100 g of thymus would increase in tissue from treated animals.

In most work reporting changes in enzyme

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TABLE I. Effect of Irradiation on Yield and RNA Content of Thymus Fractions.

Fraction	Control			Treated		
	g/100 g	mg/thymus	% RNA	g/100 g	mg/thymus	% RNA
Supernatnat A	.890	28.2	9	1.66	17.9	5.9
Sediment A	.085	1.4	7.8	.242	2.6	5.5
Supernatnat B	1.450	23.2	7.4	.767	8.3	4.9
Sediment B	.155	2.5	11.7	.067	.7	4.8
Supernatant C	.326	5.2	10.0	.271	2.9	6.4
Sediment C	.048	.8	5.9	.038	.4	4.1
Supernatant D_1	.541	8.7	9.6	.426	4.6	8.5
Sediment D_1	1.21	8.5	4.2	.709	7.7	3.0
Supernatant D_2	.270	4.2	11.6	.120	2.3	9.7
Sediment D_2	.429	2.2	5.2	.236	2.5	4.1

TABLE II. Effect of Hormone Treatment on Yield and RNA Content of Thymus Fraction.

Fraction	Control			Treated		
	g/100 g	mg/thymus	% RNA	g/100 g	mg/thymus	% RNA
Supernatant A	.839	32.4	10.2	1.445	22.6	4.5
Sediment A	.058	1.4	8.6	.184	2.9	3.9
Supernatant B	1.392	24.2	9.0	.676	10.6	5.7
Sediment B	.154	6.9	8.9	.131	2.1	3.5
Supernatant C	.467	28.4	9.4	.140	2.2	5.1
Sediment C	.188	1.5	6.2	.040	.6	2.7
Supernatant D_1	.758	47.3	9.8	.114	1.05	5.1
Sediment D_1	.802	40.6	5.5	.018	.18	3.8
Supernatant D_2	.245	10.9	10.2	.205	.2	9.2
Sediment D_2	.260	13.8	5.0	.055	.35	5.3

activities in thymus after treatment, the tissue extract is comparable to Fraction A or a mixture of Fractions A, B, and C. The change in yield and composition of Fraction A suggests that it is predominantly from the sensitive lymphocytes. The marked change in composition of this fraction as indicated by the increase in amount of serum (or lymph) proteins while the amount of cellular proteins decreases, again demonstrates the inaccuracy of expressing changes in enzyme activities in terms of nitrogen content(3).

Method II. The results obtained using this method are listed in Tables III and IV. The data indicate that S_1 is predominantly from cytoplasm of lymphocytes. The yield of S_1 is reduced when expressed as mg per thymus. Electrophoretic analysis shows a higher weight fraction of serum albumin in tissue extracts from treated animals (Fig. 1). Component 1 previously has been demonstrated to be serum albumin(7). Component 2 has been shown to contain most of the ribonucleic acid in the extract(8). The decrease in this component is also illustrated in the electrophoretic patterns and is consistent with the large drop in ribonucleic acid content of the fractions. The corresponding de-

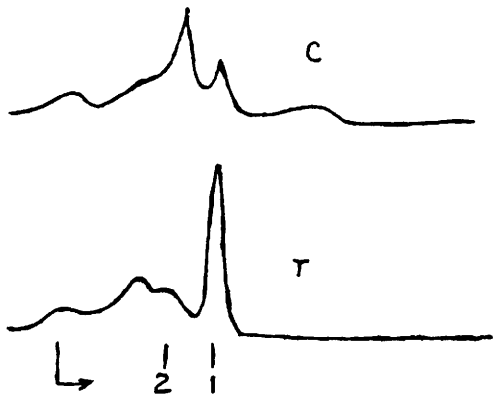

FIG. 1. Electrophoretic patterns of fraction S_1 from control (C) and cortisol-treated (T) animals. S_1 from thymus of irradiated animals is the same as that from hormone-treated animals. Patterns are of the descending limb after 120 min under a potential gradient of 4.4 volts/cm in veronal buffer, μ 0.10, pH 8.6, diagonal slit angle 45°. 1 designates the albumin and 2 fraction 5.1P.

TABLE III. Effect of Irradiation on Yield and RNA Content of Thymus Fractions.

Fraction	Control			Treated		
	g/100 g	mg/thymus	% RNA	g/100 g	mg/thymus	% RNA
S_1	2.32	46.4	8.3	2.130	15.6	3.9
Sediment S_1	.858	16.9	12.9	.879	6.4	8.1
Saline extract	.401	7.9	10.3	.766	5.6	9.2
Sed. saline extract	.122	2.4	2.5	.436	3.2	3.6
Nuclear fractions						
Supernatant A	.340	9.9	13.	.176	1.3	10.3
Sediment A	.055	.1	10.	.041	.3	8.5
Supernatant B	.320	9.3	7.7	.091	.7	6.1
Sediment B	.020	1.4	7.6	.015	.1	5.9
Supernatant D	.270	5.3	9.6	.089	.65	3.4
Sediment D	.218	4.3	4.6	.139	1.0	3.1
Nuclear residue	3.47	68.5	2.5	.928	6.9	2.8
Final residue	4.88	96.4	2.2	12.55	92.0	1.2

TABLE IV. Effect of Hormone Treatment on Yield and RNA Content of Thymus Fractions.

Fraction	Control			Treated		
	g/100 g	mg/thymus	% RNA	g/100 g	mg/thymus	% RNA
S_1	2.38	84.9	8.1	1.90	14.3	2.9
Sediment S_1	.92	33.1	12.7	.67	5.1	8.7
Saline extract	.17	6.7	8.4	.43	3.3	7.3
Sed. saline extract	.03	1.4	8.4	.23	1.8	4.4
Nuclear fractions						
Supernatant A	.28	10.5	14.0	.06	.5	7.8
Sediment A	.07	2.4	13.6	.02	.1	11.9
Supernatant B	.43	15.4	6.3	.11	.9	4.8
Sediment B	.02	.6	7.7	.003	.05	5.4
Supernatant D	.27	9.8	7.2	.01	.08	10.8
Sediment D	.14	4.8	4.8	.03	.3	11.9
Nuclear residue	4.17	152.8	2.7	1.82	14.2	2.9
Final residue	2.95	106.9	3.0	9.7	74.6	2.2

crease in total amount of nuclear material confirms that these fractions are predominantly from the sensitive lymphocytes.

The data from the saline extracts show little change in amount per thymus after irradiation suggesting that this fraction may originate from the radioresistant elements of the tissue. The increase in yield per 100 g of tissue and the fact that the ribonucleic acid in the fraction does not change also support this conclusion. The increase in yield of final residue when expressed as grams per 100 g of thymus and lack of change when expressed as mg per thymus indicates that the residue is also from radioresistant elements of the tissue.

Comparison of the data from Tables III and IV indicates some differences between the effects of hormone treatment and those resulting from irradiation. The yield in mg/thymus of saline extractable material is much lower following hormone administration. There is no change in the final residue after irradiation, whereas cortisol treatment causes a decrease in the amount of this fraction per thymus. The results suggest that the reticular components, as well as the lymphocytes, were affected by the regimen of hormone treatment. Dougherty(9) has stated that reticulum cells show no degenerative effects after a single injection of ACTH or adrenal cortical hormones. Baker *et al.*(10) investigated histological changes in lymphatic tissue of animals following prolonged treatment with adrenocorticotropin. Although lymphocytes were much more sensitive, reticulum cells were affected by adrenocortical activity. The extent of the effect varied with duration

of the treatment and dosage of hormone. Extensive changes were noted after 21 days of hormone administration.

Summary. Rabbits were exposed to 650 r of radiation or were injected with 10 mg cortisol for 3 days. Two methods were used for fractionation of thymus from control and treated animals. Method I was based on sequential extraction with water and saline. Nuclear and cytoplasmic fractions of lymphocytes were prepared by Method II. Minced tissue was stirred in sucrose-$CaCl_2$ and residual material homogenized in saline. Method I does not distinguish cytoplasmic and nuclear components. The weights of all fractions except Fraction A were lower in experimental animals when data are expressed as g/100 g thymus. A large portion of Fraction A was found to be serum proteins as determined by the large increase in albumin in electrophoretic patterns. RNA was lower in most fractions from experimental animals. Electrophoretic analysis of the S_1 cytoplasmic fraction from lymphocytes obtained by Method II, again shows a higher weight fraction of albumin in the extract from treated animals. Decreases in fractions attributed to nuclear material were noted in treated animals. Nuclear fractions were attributed largely to lymphocytes. Saline extracts and final residue show little change following irradiation, indicating these fractions are from radioresistant elements. Both fractions are more sensitive to hormone treatment.

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Immunochemical Studies on Kidney Hypertrophy of the Rat. (27881)

R. HIRAMOTO,* J. BERNECKY AND J. JURAND*
Department of Biochemistry Research, Roswell Park Memorial Institute, New York State Department of Health, Buffalo

It has been the contention that in conditions of unilateral nephrectomy the hypertrophy of the remaining kidney occurs with no increase in the number of glomeruli as made by counting studies(1,2). This hypertrophy begins almost immediately after nephrectomy and some correlation with an increase in mitotic activity in the remaining kidney has been reported(3,4).

We have observed that in newborn animals treated with rabbit anti-rat kidney serum rapid localization of anti-kidney antibodies was seen in all glomeruli, by means of the indirect fluorescent antibody technic. Therefore, the antibody can be used as a marker for glomeruli. When animals treated in this manner are allowed to grow to 6 weeks before being sacrificed the newly formed glomeruli that have become active subsequent to the treatment are unmarked and hence can not be stained for the presence of the marker antibody. Only those glomeruli that were available to the antibody at time of treatment can be stained(5). Since every glomerulus can be marked by the antibody the procedure would be a sensitive way of

* Present address: St. Jude Hospital, Memphis, Tenn.