with one of us (H.A.T.) in his laboratories.

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Differential Extraction of Lymphocytic and Reticulum Cell Fractions from Thymus.* (27879)

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

Most fractionation schemes place little emphasis on quantitative aspects of tissue composition. A procedure for qualitative and quantitative extraction of thymus has been reported(1). The method does not, however, distinguish between lymphocytic and reticulum cells, the 2 predominant cell types in thymus. Since these 2 cells differ in their sensitivity to hormone treatment and irradiation(2,3) a fractionation procedure which differentiates between lymphocytic and reticular components is desirable. The following report describes methods which are developed for this purpose. One of the procedures permits separation of cytoplasmic and nuclear components from lymphocytes. A subsequent paper(4) concerns the effect of ionizing radiation and cortisol treatment on the fractions obtained by these methods.

Methods. Calf thymus or thymuses from male rabbits, 3 to 4 months old, were used. The tissue was chilled and immediately fractionated by one of the methods described below. All operations were carried out at 3-4°C. The discussion stresses the data obtained from rabbit thymus since the results can be expressed more reliably as yield per thymus. Some variation in the quantity of fractions was observed. These differences could be correlated with the condition of the thymus in the experimental animals and did not alter interpretation of the data.

A. Method I. The procedure is based on sequential extraction. Thymus was minced, put through an onion press and stirred in water for 10 minutes (10 ml/g wet weight). The supernatant after centrifugation at 8500 $\times$ *g* for 4 minutes was designated as the water soluble Fraction A. The sediment from the preceding step was stirred for 10 minutes in a volume of 0.3 M NaCl equal to the volume of the sediment. The supernatant after centrifugation at 8500 $\times$ *g* for 4 minutes was called Fraction B. Re-extraction of the sediment with 0.15 M NaCl yielded a small amount of saline soluble material with the same characteristics as those of Fraction B. Finally, the sediment from the third step was homogenized in 0.15 M NaCl in a Waring blendor at top speed for 1 minute. The resulting brei was centrifuged at 8500 $\times$ *g* for 4 minutes. Homogenization was repeated 4 times. The combined 8500 $\times$ *g* supernatants were designated as Fraction D. The 8500 $\times$ *g* supernatants from each fraction were centrifuged at 23,000 $\times$ *g* for 1 hour to obtain the corresponding supernatant and sediment fractions. The insoluble ma-

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TABLE I. Dry Weight and Ribonucleic Acid Content of Rabbit Thymus Fractions Obtained by Method I.

Fraction	mg/thymus	g/100 g	% RNA
A	13.2	.825	9.0
Sed A	1.4	.085	7.8
B	23.2	1.45	7.4
Sed B	2.5	.155	11.7
C	5.2	.326	10.0
Sed C	.8	.048	5.9
D_1	8.7	.541	9.6
Sed D_1	19.5	1.21	4.2
D_2	4.2	.270	11.6
Sed D_2	7.0	.439	5.2

terial remaining after homogenization was called final residue.

The lyophilized dry weights of the non-dialyzable material in each fraction, expressed as grams per 100 g wet weight of thymus and as yield per thymus, are shown in Table I. Ribonucleic acid values as determined by the method of Schmidt and Tannhauser(5) are also shown.

B. Method II. A fractionation method based on the procedure of Allfrey, Mirsky and Osawa(6) distinguished more clearly between nuclear and cytoplasmic fractions from lymphocytes and also between constituents from lymphocytic and reticulum cells. Minced tissue was put through an onion press, and stirred in a Waring blendor with 9 volumes of 0.25 M sucrose-0.0033 M $CaCl_2$ at approximately 1000 rpm for 4 minutes. The mixture was filtered through gauze and then double napped flannelette. The filtrate was centrifuged at 1400 $\times$ *g* for 7 minutes. The above steps were repeated on the material left on the gauze using one-half the original volume of sucrose-$CaCl_2$ mixture. The two 1400 $\times$ *g* sediments, the nuclear fractions, were pooled and washed twice with sucrose-$CaCl_2$. The 1400 $\times$ *g* supernatants were also pooled and centrifuged for 1 hour at 23,000 $\times$ *g* to obtain a supernatant cytoplasmic fraction, referred to as S_1, and a sediment, sed S_1.

The residual tissue on the gauze was removed and homogenized in 0.15 M NaCl at top speed in a Waring blendor for 15 seconds (2 ml 0.15 M NaCl per gram wet weight of original starting material). The mixture was centrifuged at 8500 $\times$ *g* for 4 minutes and the extraction repeated on the sediment. The two 8500 $\times$ *g* supernatants were combined and centrifuged for 1 hour at 23,000 $\times$ *g* to obtain a saline extract supernatant and sediment. The remaining material after saline extraction is called the final residue.

It was also of interest to disrupt the nuclei and determine the amount of non-nucleohistone material. Washed nuclei, when stirred in water for 15 minutes, swell markedly and form a gel. Centrifugation at 8500 $\times$ *g* for 4 minutes yields a supernatant, water soluble Fraction A. The sediment is extracted by stirring 10 min with a volume of 0.3 M NaCl equal to the volume of the sediment. The supernatant obtained after centrifugation at 8500 $\times$ *g* for 4 minutes is called Fraction B. Additional saline soluble material was extracted by stirring in 0.15 M NaCl a second time. The residue was homogenized twice at top speed in a Waring blendor in 0.15 M NaCl (1 ml/g starting tissue) to obtain Fraction D. The final residue is predominantly deoxyribonucleoprotein. Supernatants from each fraction were centrifuged at 23,000 $\times$ *g* for 1 hour.

All fractions were dialyzed and lyophilized to obtain dry weights. The fractions were analyzed for ribonucleic acid using the method of Schmidt and Tannhauser(5).

Results and discussion. Results from the sequential extraction technic show that over half of the material extracted from the thymus was solubilized by slow stirring and saline. More drastic homogenization with saline yields an additional 1.5 to 2 g per 100 g. The residue after extraction of Fraction D contains the deoxyribonucleoprotein of the tissue. All the fractions except sediment C_1, D_1, and D_2 contain about 10% ribonucleic acid.

Most data in the literature concerning thymus describe fractions which consist of a mixture of fractions, A, B, C and D depending on the extent of homogenization and the media used. It is apparent that this type of procedure will not give definitive information pertaining to soluble nuclear and cytoplasmic components. Results from Method II support the conclusion that Fractions A, B and C are predominantly from the lymphocytes

TABLE II. Dry Weight and Ribonucleic Acid Content of Rabbit Thymus Fractions Obtained by Method II.

Fraction	mg/thymus	g/100 g	% RNA
Cytoplasmic fractions			
S_1	45	1.85	8.6
sed S_1	20.3	.831	14.9
Nuclear fractions			
A	4.4	.182	11.8
sed A	1.3	.055	9.9
B	4.8	.196	6.6
sed B	.3	.012	7.4
D	2.0	.081	10.2
sed D	1.8	.075	4.6
Residue	63.0	2.56	2.5
Saline extract	14.9	.611	10.3
sed saline extract	12.9	.531	3.4
Final residue	120	4.4	2.2

while Fraction D is a mixture of material from the nuclear residue and the reticular elements of the tissue.

Nuclear and S_1 fractions, as obtained using Method II, are considered as predominantly lymphocytic in origin and the saline extract as largely from reticulum cells. These conclusions are supported by additional findings discussed in the accompanying paper. Data in Table II indicate that the greatest part of the extractable material in thymus is found in the S_1 fraction which is obtained by slow stirring with sucrose-$CaCl_2$. As calculated by Allfrey *et al.*(7) the volume fraction occupied by the nucleus in the lymphocyte is 61% of the volume of the cell. The yield of cytoplasmic material in fraction S_1 (65 mg/thymus) and the amount of total nuclear material (78 mg/thymus) agrees well with this calculation.

The amount of extractable material in the nuclear fractions, other than deoxyribonucleohistones represents only 20% of the dry weight of the total nuclear fraction. This indicates that if the nuclei are ruptured by a fractionation procedure, such as homogenization in water or saline, the yield of the cytoplasmic fractions might not be noticeably affected. However, contamination by nuclear enzymes would be a serious consideration in biochemical studies.

It can also be seen that even after extensive homogenization of the tissue remaining after the sucrose-$CaCl_2$ extraction, only 1 g per 100 g thymus of material was obtained (saline extract). Excluding the initial nuclear fractions approximately 70% of the extractable material in thymus, therefore, was found in the initial sucrose-$CaCl_2$ extract, the lymphocytic fraction. Drastic homogenization is not needed, therefore, to obtain cell fractions from lymphocytes of thymus.

Table II also indicates the distribution of ribonucleic acid in the various fractions. The value for the nuclear residue is in accord with that reported by Allfrey *et al.*(7) for nucleohistone.

Method II should be a valuable procedure for studying nuclear and cytoplasmic relationships in the lymphocytes. In addition, this method of fractionation should be helpful in analysis of the changes in composition of the thymus after x-radiation or hormone treatment. A subsequent paper describes the effect of such agents on the yield and composition of the various fractions.

Summary. Two methods are described for the fractionation of thymus. Results using Method I indicate that sequential extraction with water or saline does not provide information distinguishing nuclear and cytoplasmic relationships. Data from Method II demonstrate that nuclei and cytoplasmic fractions of lymphocytes are obtained by slow stirring in sucrose-$CaCl_2$. The cytoplasmic fraction amounts to approximately 40% of the mass of the cell. Only 20% of the dry weight of the nuclei is non-nucleohistone material. Homogenization of residual tissue at top speed in a Waring blendor results in only 15% more material extracted.

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