The Chemical Fractionation of Rabbit and Swine Thymus.* By Eugene L. Hess and Saima E. Lagg. (*From The Rheumatic Fever Research Institute, Chicago.*)‡

A previous report described a generalized method for the chemical fractionation of lymphatic organs (1). It was shown that the method was applicable to bovine thymus and to ovine and bovine palatine tonsils. The study has now been extended to include two additional species, in order to ascertain the general applicability of the method, and also to include an animal common to laboratories. The procedure would be much more useful if applicable to the thymus of a small mammal such as the rabbit, since relevant biological studies become more feasible, and numerous biological problems could be undertaken using data from normal animals as a frame of reference. Problems such as the radiation sensitivity of lymphoid tissue (2), the antibody content of lymphatic organs (3), and the changes in composition resulting from the involution of the thymus under conditions of stress (4), appear to be susceptible to study using chemical fractionation procedures. It is the purpose of this report to point out that the fractionation procedure previously described (1) has been found equally applicable to porcine and rabbit thymus.

Materials and Methods

The thymus glands were procured from young animals, packed in ice, and the extraneous tissue removed within 2 hours from the time of slaughter. All experimental operations have beeen discussed previously (1, 5). The preparation of extracts and symbols used to represent the various fractions are the same as used previously (1). The term optical concentration was defined in the previous study (1).

In our experience rabbit thymus contained a much larger amount of fat dispersed throughout the organ than did porcine and bovine thymus. This was evident even with young rabbits carefully selected with respect to age and size. Altogether from 100 gm. of starting material, representing the thymus glands from 18 rabbits, 42 gm. of fat were removed by hand from cold thymus and an additional 11 gm. floated to the surface of the extracts after centrifuging. As a consequence only 47 gm. of non-fat–containing material was used in the rabbit experiment. The results have been expressed, however, in terms of 100 gm. wet tissue containing no fat.

RESULTS

As can be seen in Fig. 1 *A* the electrophoretic pattern obtained from the total extract (E_T) using rabbit thymus was indistinguishable from the previously published pattern (1) from bovine thymus. The pattern from hog thymus is virtually identical with that seen in Fig. 1 *A*.

In each subsequent fraction examined (5.1 P, 5.1 S, 3.0 P, 3.0 S, 6.2 P, 6.2 S, 4.7 P and 4.7 S) the electrophoretic patterns closely resembled those published in the earlier study (1). For comparison purposes patterns obtained using the PNA-type nucleoprotein fraction 3.0 P and fraction 4.7 S from hog thymus are shown in Fig. 1 *B* and 1 *C* respectively.

In all cases yields of each fraction, on a dry weight basis, were comparable to those reported in the earlier study (1). The optical concentrations and yields are listed in Table I. The materials remaining insoluble after three extractions

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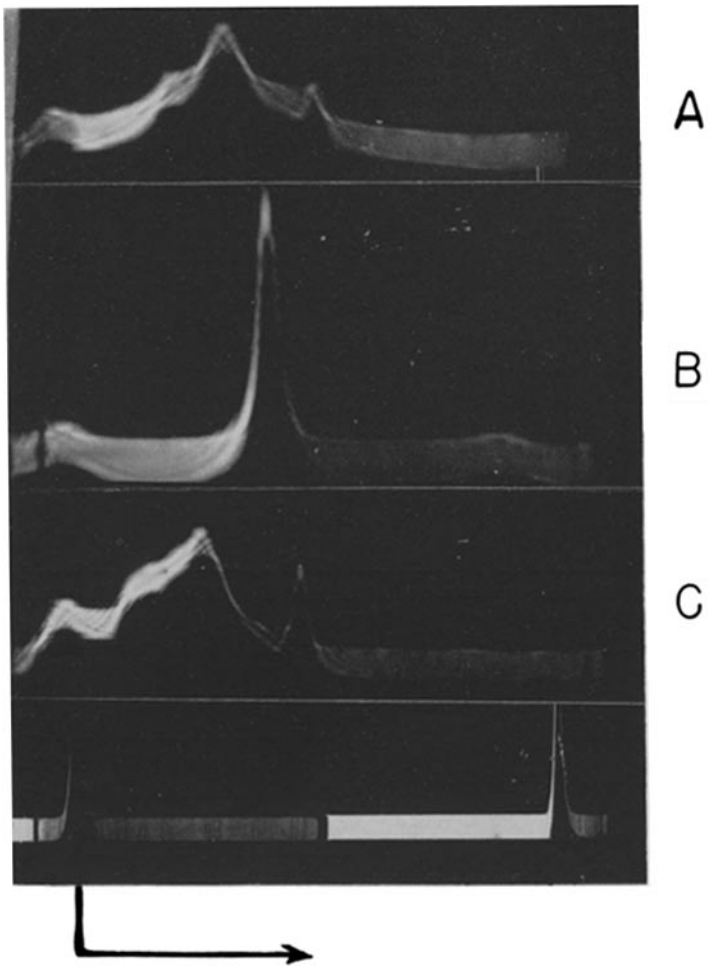

FIG. 1. Electrophoretic patterns (descending limb) from thymus extracts, after 120 minutes under a potential gradient of 6.4 volt cm.$^{-1}$ in veronal buffer μ 0.10, pH 8.6. Magnification factor from cell to print is 1.02. Ascending patterns are essentially enantiographs of the patterns shown. *A*. Pooled extracts of rabbit thymus, protein concentration approximately 0.85 per cent, diagonal slit angle 50°. *B*. Fraction 3.0 P (PNA-nucleoprotein) from swine thymus. Protein concentration approximately 0.6 per cent, diagonal slit angle 60°. *C*. Fraction 4.7 S from swine thymus. Protein concentration approximately 1.2 per cent, diagonal slit angle 40°.

amounted to 9 gm. as in the case of the earlier study (1).

TABLE I

Optical Concentrations (O. C.) and Yields of Hog and Rabbit Thymus Extracts and Fractions per 100 gm. Wet Tissue

Fraction	Swine			Rabbit		
	*Optical concentration 260 mμ	Optical concentration 280 mμ	Yield	Optical concentration 260 mμ	Optical concentration 280 mμ	Yield
			gm.			*gm.*
E_T	32.2	20.5		27.4	17.5	
E_{TA}	17.2	13.5	4.7	15.7	12.0	4.1
5.1 P			3.20			2.7
3.0 P			1.80			1.40
3.0 S			1.10			1.01
6.2 P			0.78			
6.2 S			0.25			
5.1 S	2.41	2.88	1.45	2.31	2.61	1.20
4.7 P			0.18			
4.7 S			1.23			
Residue‡						8.9

* Optical concentration represents optical density times dilution at the wave length specified.

‡ Chiefly nuclei and connective tissue remaining insoluble after three extractions with 0.15 M NaCl.

DISCUSSION

The chief point of interest in the above study was to compare the macromolecular composition of thymus from several animal species. It is of interest, both from an academic and a pragmatic viewpoint, that according to the chemical and physical methods employed in this study and in the earlier report (1) hog, calf, and rabbit thymus extracts were indistinguishable. One can infer that the chemical fractionation procedure employed would be applicable to thymus from other common mammals. It also seems reasonable to suggest, as has been shown for palatine tonsils and thymus, that the method may apply to other lymphatic tissue as well. The fractionation procedure can, therefore, be recommended as a possible tool for biological problems where macromolecular composition is relevant to evaluation of results. The composition and yield data given above for rabbit thymus provide a convenient frame of reference applicable to a small mammal readily available in most laboratories.

The absence of color when rabbit and hog thymus extracts were heated with diphenylamine reagent provides further evidence that the mucoprotein found in palatine tonsil extracts was not a lymphocytic constituent (1, 5).

SUMMARY

A chemical fractionation procedure, previously found applicable to bovine thymus and bovine and ovine palatine

tonsils, was used to fractionate rabbit and hog thymus. With respect to the chemical fractionation steps, yields of fractions, and optical and electrophoretic properties, extracts from hog and rabbit thymus were indistinguishable from similar extracts prepared from calf thymus. The study provides composition and yield data applicable to the thymus of a small mammal readily available in most laboratories.

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