THE CHEMICAL FRACTIONATION OF LYMPHATIC ORGANS*

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INTRODUCTION

If the lymphatic tissue found in abundance in animals were assembled into one organ, this gland would be one of the larger ones of the body. Despite both the ubiquity and quantity of this tissue in the animal body relatively little is known about either its composition or its function.

As early as 1887 Halliburton studied extracts of lymphatic organs obtained from cats and dogs. He concluded from these studies that at least five components were present in the extract (1). Deoxyribonucleoproteins have been isolated from thymus (2–5) and characterized as nucleohistones (6, 7). More recent studies have shown that these proteins are complex mixtures (5, 8, 9). A number of workers have made electrophoretic examinations of extracts from various lymphatic organs (10–14). White and coworkers (11, 15, 16) have presented evidence suggesting that lymphatic organs are depots, or possibly even the source, of the immune globulins of serum.

Work on the chemical fractionation of lymphatic organs was initiated in this laboratory seven years ago. The isolation of a mucoprotein (13) and an albumin (17) from bovine palatine tonsils has been reported. A simple protein with a molecular weight of approximately 2.3×10^6 has been isolated from bovine palatine tonsils and characterized (18). This protein showed remarkable sensitivity to ultraviolet radiation (19). The pentose type nucleoproteins have been isolated from the same organ and partially characterized (20). These nucleoproteins amount to about 40 per cent by weight of the original extract. The above four proteins represent the major components present in the 0.15 M NaCl extracts of the tonsil and account for about 65 per cent, by weight, of the proteins in the extract. Evidence has been presented for the presence of a proteolytic enzyme with a maximal activity at pH 3 in extracts of bovine palatine tonsils (21).

A general fractionation procedure will be presented in this report. It will be shown that this procedure, designed for bovine palatine tonsils, is applicable to sheep palatine tonsils and bovine thymi, as well. In some instances chemical, physical, and biological properties of the various fractions will be discussed.

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Experimental

The method of procuring bovine tonsils has been described previously (13). The same conditions were complied with in the procurement of sheep tonsils. Bovine thymus was excised from freshly slaughtered young calves, packed with ice, and the extraneous tissue removed within 2 hours of slaughter. The gland was cut into small pieces, rinsed in 0.15 M NaCl, and stored at −40°C. until used. Other experimental procedures have been described (13).

All fractionation operations were carried out in a cold room held at 2°C. Except for centrifugation steps, the tissue and the various fractions were continuously maintained at a temperature less than 5°C. The temperature of solutions centrifuged in the cold room for 60 minutes at 20,000 *g* rose approximately 10°C.

Extraction

Fractionation steps were carried out commencing with 100 gm. batches of the cleaned, rinsed tissue.[1] The glands were homogenized in a Waring blendor at high speed for 15 seconds using 200 ml. of 0.15 M NaCl. As discussed previously (13), the time of homogenization was a critical step in the procedure. The homogenate was stirred for 30 minutes and then centrifuged for 60 minutes at 20,000 *g* in an SS-1 Servall angle-head centrifuge. The supernatant solution from the first extraction, called E_1, had a pink color due to traces of hemoglobin.

The sediment from the first extract was suspended in 200 ml. of 0.15 M NaCl, homogenized 15 seconds at high speed, stirred, and centrifuged as before. The 200 ml. of straw-colored supernatant solution was called E_2. The above operation was repeated a third time yielding supernatant solution E_3.

Aliquots from each extract were removed for optical density measurements and the diphenylamine test (13). The average values for 60 separate fractionation experiments with bovine tonsils as well as for the fractionation of sheep tonsils and bovine thymus are listed in Table I.

The three supernatant solutions E_1, E_2, E_3, were combined giving about 600 ml. of solution, containing in the case of bovine tonsils approximately 5 to 5.5 gm. of non-dialyzable dissolved proteins. An aliquot of the combined supernatant solutions, called E_T, was also removed for optical density measurements, the diphenylamine test, and for the electrophoretic examination. According to Mirsky and Pollister (5) the soluble constituents represent to a large extent at least, the cytoplasmic constituents of the cells.

The combined extracts were dialyzed for about 42 hours against four changes of distilled water to essentially zero ionic strength. Approximately 1.8 gm. of dialyzable materials, in addition to sodium chloride, was present. Therefore about 6.8 to 7.3 gm. of dissolved material was originally present in the case of bovine tonsils, in the first three extracts. The dialyzable substances, when examined in the spectrophotometer, absorbed strongly and showed maximum absorption at 260 mμ suggesting the presence of considerable amounts of purines and pyrimidines. The combined dialyzed extract will hereafter be designated fraction A.

The materials remaining insoluble after three extractions consisted chiefly of nuclei

[1] The 100 gm. wet tissue represents an average of about 14.5 gm. dry weight of non-dialyzable materials.

and connective tissue. This material amounted to approximately 9 gm. and was discarded.

TABLE I

Optical Concentrations (OC) of the Various Extracts of Lymphatic Organs

Tissue	Extract*	Non-dialyzable solids (dry weight)	O.C.‡ 260 mμ	O.C‡ 280 mμ	O.C.‡ diphenylamine reaction
		gm.			
Bovine tonsils (BT)	E_1	3.0 ± 0.4	36.8 ± 7.7	30.5 ± 5.8	2.9 ± 0.7
	E_2	1.5 ± 0.3	21.2 ± 2.5	17.5 ± 2.0	2.4 ± 0.5
	E_3	0.8 ± 0.2	12.5 ± 1.6	10.0 ± 1.5	1.4 ± 0.4
	E_T		22.9 ± 2.6	18.7 ± 1.7	2.6 ± 0.7
	E_{TA}	5.3 ± 0.4	15.8 ± 2.0	13.4 ± 1.5	2.1 ± 0.5
Sheep tonsils (ST)	E_1		38.4	26.4	2.3
	E_2		16.1	11.0	1.7
	E_3		6.9	4.8	0.7
	E_T		20.1	13.9	1.6
	E_{TA}	5.0	14.1	11.1	—
Bovine thymus (BTh)	E_1	2.9	66.2 ± 5.5	45.7 ± 2.8	None
	E_2	1.0	23.2 ± 4.3	15.6 ± 2.7	
	E_3	0.5	9.6 ± 2.2	6.3 ± 1.4	
	E_T		31.9 ± 2.9	21.9 ± 1.5	
	E_{TA}	4.4	16.7 ± 1.8	13.1 ± 1.4	
BT fraction 5.1 S		1.8 ± 0.3	3.6	4.2	1.8 ± 0.4
BTh " 5.1 S		1.4 ± 0.2	2.8 ± 0.4	3.4 ± 0.4	None

* Additional extractions yielded further soluble materials up to approximately 8.5 gm./100 gm. tonsil for seven extractions, representing about 53 per cent of the dry weight of the gland. The material obtained in extraction steps 4 to 7 resembled the material obtained in the precipitate (5.1 P) at pH 5.1 (*vide infra*). In the work reported here only the first three extracts were used. T refers to the combined total extract before dialysis and TA after dialysis. The above data are all based upon 100 gm. of fresh tissue.

‡ Optical concentration represents the optical density times the dilution at the wavelength specified. The last column represents the optical concentration read at 530 mμ with the diphenylamine test (22). In this instance 1 ml. of extract from the organ was diluted to 10 ml. with water. To 1.5 ml. of this solution was added 3.0 ml. of "sensitive" (22) diphenylamine reagent and the mixture was heated for 30 minutes on a boiling water bath. The mixture was cooled in ice water, allowed to come to room temperature, and the optical density was read in a Model DU Beckman spectrophotometer.

Fractionation of Extracts[2]

The electrophoretic pattern from fraction A obtained from bovine thymus (BTh) is shown in Fig. 1 A. Bovine tonsils (BT) and sheep tonsils (ST) give

[2] The weight values listed refer to dry weights based upon solutions dialyzed against water and lyophilized. All weights refer to bovine palatine tonsil fractionations for which we have

essentially the same electrophoretic patterns. A schematic representation of the fractionation procedure is shown in Fig. 2.

Step 1.—The first step in the fractionation was designed to separate the nucleoproteins from the other proteins in the solution. The ionic strength of the solution was adjusted to 0.025 with NaCl and the pH was lowered to 5.1 using 0.1 N acetic acid. The precipitate amounted to approximately 3.7 gm. and was collected by centrifugation. Approximately 1.8 gm. of solids (dry weight) remained in the supernatant solution. The precipitate from this step was called 5.1 P and the supernate was called 5.1 S. The electrophoretic pattern given by the precipitate from BTh (5.1 P) is shown in Fig. 1 B. Essentially the same patterns were obtained when fraction 5.1 P from BT and ST were examined in the Tiselius apparatus. The corresponding pattern given by the supernatant solution (5.1 S) may be seen in Fig. 1 C. The ultraviolet absorption behavior as well as chemical analyses disclosed that fraction 5.1 S was devoid of nucleic acids whereas fraction 5.1 P was rich in nucleic acid. When fraction 5.1 P was tested at a concentration of 1 mg./ml. with the diphenylamine reagent of Dische (23) a colorless solution was obtained from BTh and ST. Since a blue color with a maximum absorption at 600 mμ is indicative of the presence of deoxypentoses, it was concluded that the nucleic acid present in 5.1 P is the pentose type.

Fraction 5.1 P from bovine tonsils usually gave a slight purple color with the Dische reagent and a maximum in the absorption curve at 530 mμ, indicating that some mucoprotein (13) had coprecipitated with 5.1 P. Fraction 5.1 P contained all of the nucleic acid present in the initial extract.

Step 2.—Fraction 5.1 P was dissolved in 250 ml. of distilled water by the cautious addition of approximately 40 ml. of 0.05 N sodium bicarbonate.[3] After 5.1 P was dissolved traces of suspended particles, mostly lipide in nature, were removed by filtering through glass wool. The solution was dialyzed for 48 hours against three changes of μ 0.10 sodium monochloracetate at pH 3.0–3.05. The stringy precipitate which formed consisted chiefly of a pentose type (PNA) nucleoprotein, and has been called fraction 3.0 P. This precipitate was dissolved in 400 ml. water with the cautious addition of 0.05 M $NaHCO_3$ to pH 7. After stirring for an hour at pH 7 the precipitate dissolved to yield a somewhat turbid solution. The solution was dialyzed overnight

assembled the largest amount of information. Bovine thymus fractions in general gave somewhat smaller yields, due to a lesser amount of dissolved material in the initial extracts. In the case of the thymus, small and variable amounts of fat were present. The fat, which varied from 2 to 10 gm., floated on the surface of the centrifuged extract and was removed by filtration through glass wool.

[3] Our earlier efforts to redissolve fraction 5.1 P were uniformly unsuccessful. Some of the proteins contained in this fraction were found to be thermally labile, very sensitive to localized excess of acid and base, and to become insoluble on exposure to ultraviolet light. Furthermore the solubility of the fraction was found to be inversely proportional to the ionic strength of the system. The bicarbonate solution was added to the aqueous suspension by means of a burette, the tip of which was drawn to a fine capillary. The tip of capillary was placed under the surface of the solution. Efficient stirring was also found to be desirable. With the above precautions we have not failed to redissolve this precipitate in some 90 subsequent fractionations. Our experience with this fraction resembled what Barum and Huseby (see p. 19, reference 26) observed when studying mouse liver microsomes.

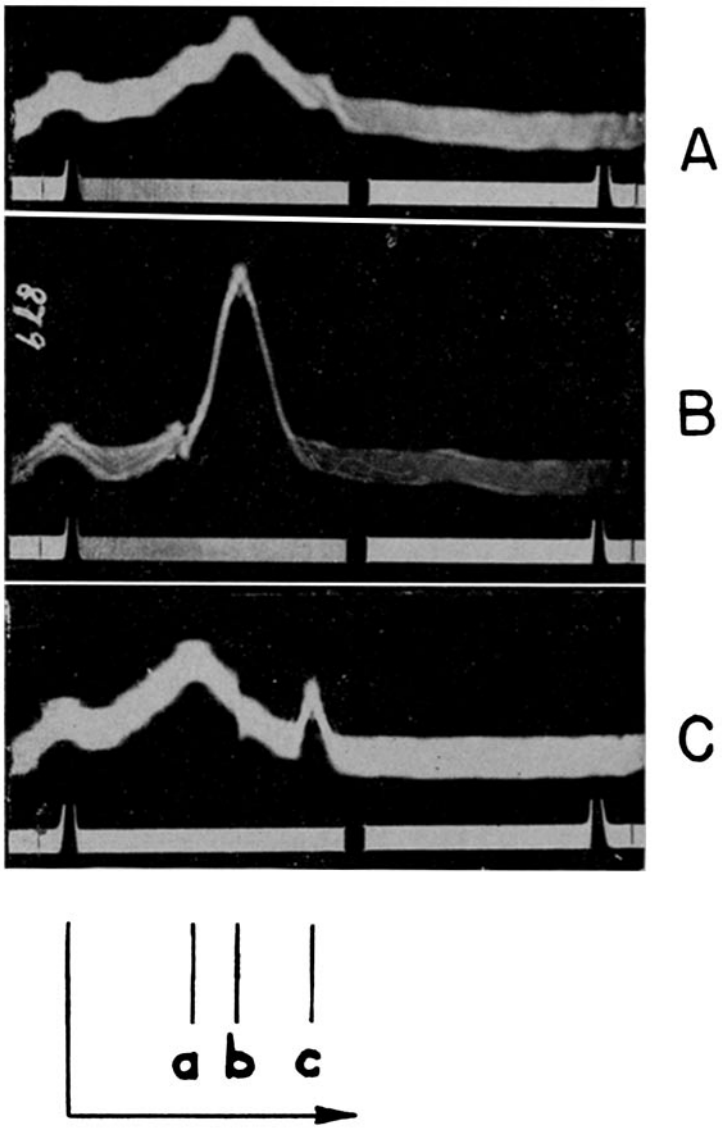

FIG. 1. Electrophoretic patterns (descending limb) from bovine thymus extracts, after 120 minutes under a potential gradient of 6.4 volt cm.$^{-1}$ in veronal buffer μ0.10, pH 8.6, diagonal slit angle 40°. Magnification ratio from cell to print is 1.02. The ascending patterns are essentially enantiographs of the patterns shown.

A, original extract (fraction A), protein concentration approximately 0.8 per cent. The mobilities of peaks labelled *a*, *b*, *c*, are -3.8, -4.9, and -6.7×10^{-5}, cm.^{2} sec.$^{-1}$ volt^{-1} respectively.

B, fraction 5.1 P, protein concentration approximately 1.0 per cent. u $= -4.9 \times 10^{-5}$ cm.^{2} sec.$^{-1}$ volt.$^{-1}$

C, fraction 5.1 S, protein concentration about 1.1 per cent. The mobility of peaks labelled *a* and *c* are -3.8 and -6.7×10^{-5} cm.^{2} volt^{-1} sec.$^{-1}$ respectively.

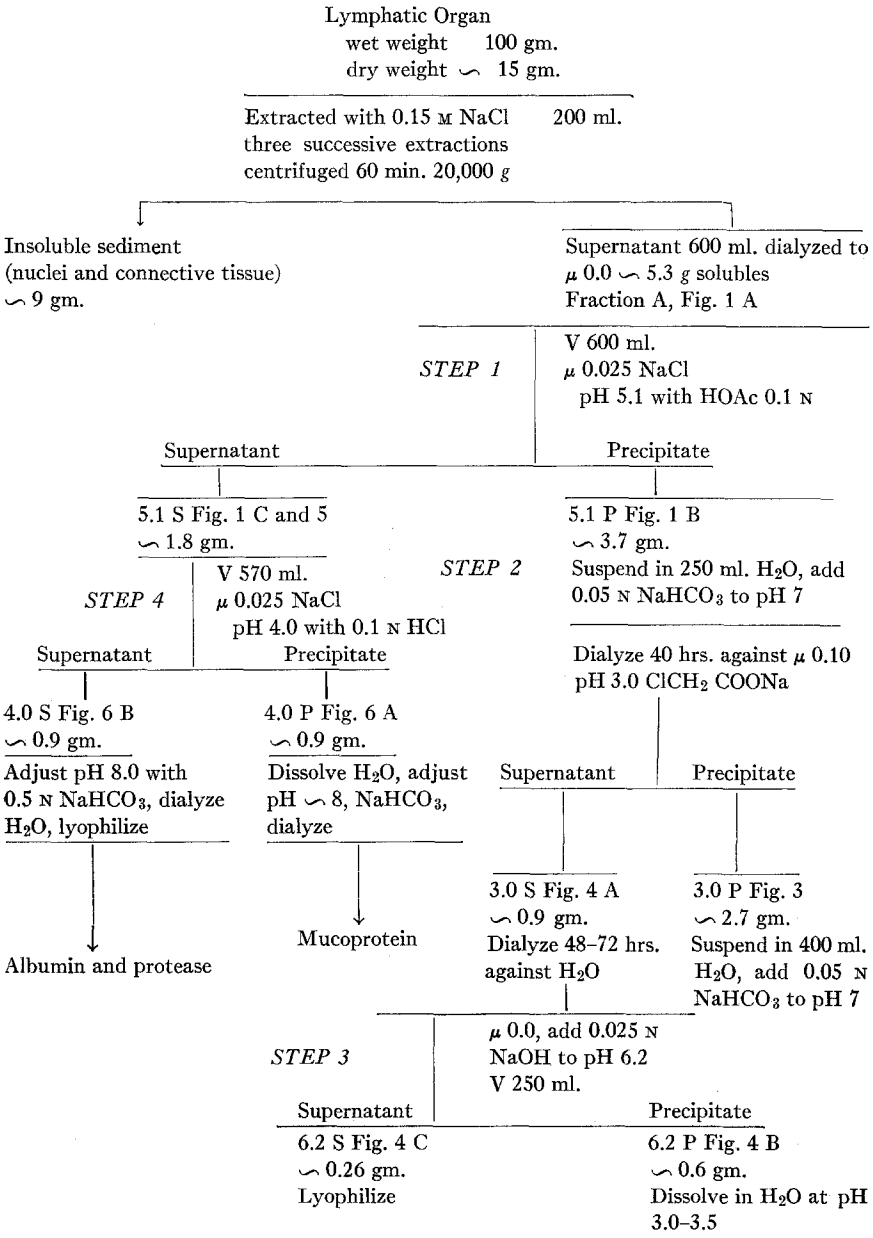

FIG. 2. Schematic fractionation procedure for lymphatic organs.

against water and as the ionic strength approached zero the turbidity departed. The solution remained quite opalescent when viewed at 90° to incident light. This striking capacity to scatter light suggested that the dissolved nucleoprotein possessed a high

molecular weight. When centrifuged 30 minutes at 20,000 *g* 85 per cent of the contents of the solution remained in the supernate. The 3.0 P from BT, BTh, and ST gave essentially the same electrophoretic patterns and a similar yield of dry material; approximately 2.7 gm. The electrophoretic pattern obtained from an experiment using bovine thymus can be seen inFig. 3. Fraction 3.0 P contained about 20 per cent of material extractable with 3:1 ethanol ether, presumably lipides, and 15 per cent PNA type nucleic acid. About 50 per cent of the lipide material was soluble in acetone. The acetone insoluble residue dissolved in alcohol and was therefore probably largely phosphatides.

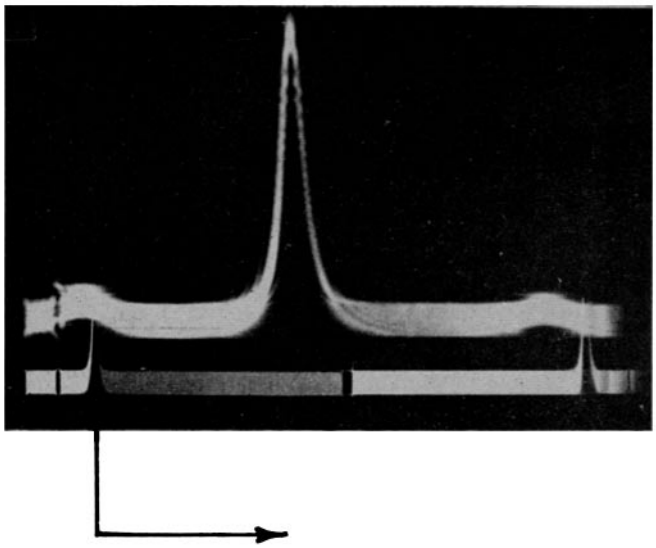

FIG. 3. Electrophoretic pattern (descending limb) of 3.0 P from bovine thymus after 120 min. in pH 8.6, μ0.10 veronal buffer under a potential gradient of 6.4 volt cm.$^{-1}$ Protein concentration approximately 0.8 per cent; diagonal slit angle 30°. Mobility -5.5×10^{-5} cm.^{2} volt^{-1} sec.$^{-1}$ Ascending and descending patterns were essentially enantiographic.

Step 3.—The supernatant solution (3.0 S) obtained at μ 0.10 pH 3.0 with monochloracetate was dialyzed for 72 hours against several changes of water. About 1 gm. of dissolved material was found to be present in this fraction. In the case of BT, BTh, and ST similar patterns were obtained in the Tiselius apparatus. In Fig. 4 A a representative pattern is shown.

Fraction 3.0 S was no longer completely soluble in veronal buffer at pH 8.6. The insoluble portion was found to be component 6.2 P (*vide infra*). Because of the solubility factor fraction 3.0 S was analyzed at pH 3.0 in phosphate buffer. A pattern similar to that seen in Fig. 4 A was also obtained in μ 0.10, pH 3.0 sodium monochloroacetate buffer. The significance of the solubility behavior is discussed below.

Three maxima were observed in the turbidity pH curve (24) obtained with this fraction, the largest of which occurred at pH 6.2. The pH of the solution was accordingly raised to 6.2 and the precipitate was removed by centrifugation. BT, BTh, and

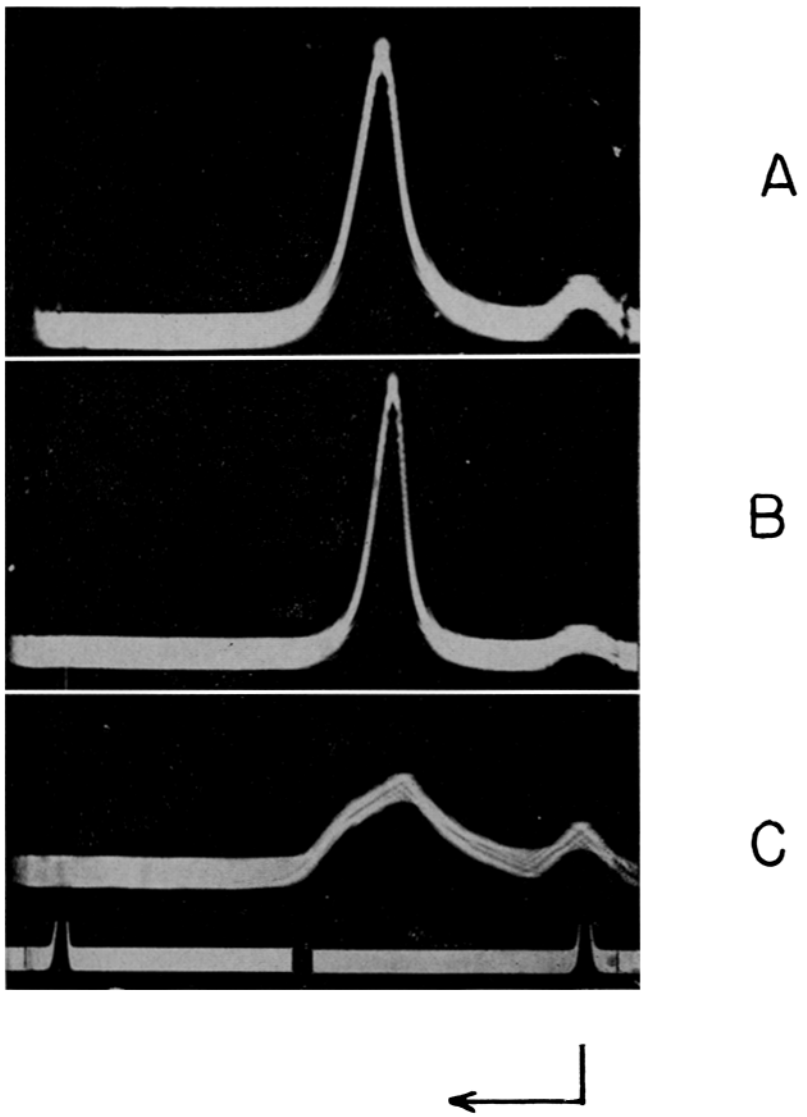

FIG. 4. Electrophoretic patterns (descending limb) of bovine tonsil fractions after 120 minutes in μ0.10, pH 3.0 sodium phosphate under a potential gradient of 5.6 volt cm.$^{-1}$

A, fraction 3.0 S protein concentration 1.3 per cent; diagonal slit angle 35°.

B, fraction 6.2 P^4 protein concentration about 1 per cent; diagonal slit angle 35°. u = 6.1 $\times$ 10^{-5} cm.^{2} volt^{-1} sec.$^{-1}$.

C, fraction 6.2 S protein concentration 1.3 per cent; diagonal slit angle 35°.

ST all behaved in a similar manner. The precipitate called 6.2 P amounted to approximately 750 mg. and gave the electrophoretic pattern seen in Fig. 4 B.[4] When the supernatant solution, 6.2 S, was lyophilized about 200 mg. of dry material was obtained. The electrophoretic pattern obtained from fraction 6.2 S is shown in Fig. 4 C.

Fraction 6.2 P, which after one additional purification step has been called component L 3, was found to be a simple protein with a molecular weight of about 2.3×10^6

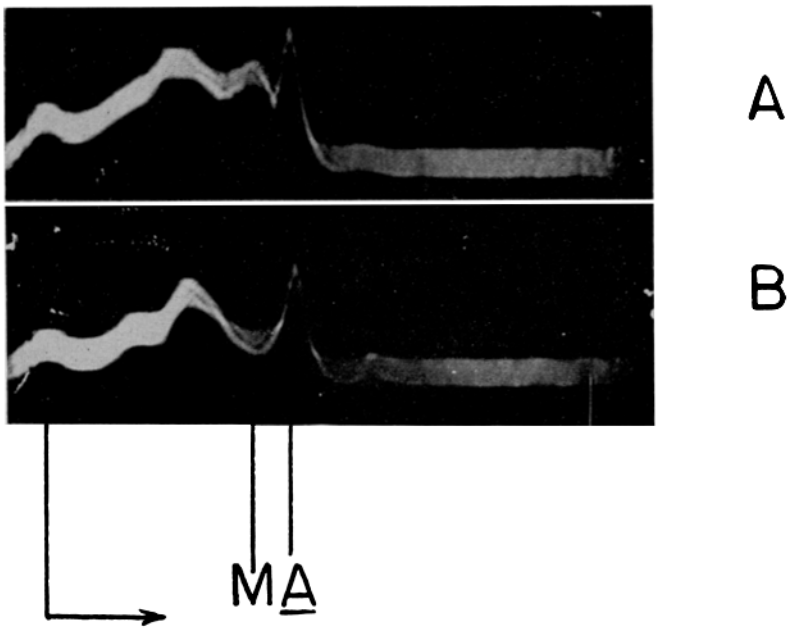

FIG. 5. Electrophoretic patterns (descending limb) of fraction 5.1 S after 120 minutes under a potential gradient of 6.4 volt $cm.^{-1}$ in veronal buffer $\mu 0.10$, pH 8.6, protein concentration 1.1 per cent; diagonal slit angle 35°. *M* designates the mucoprotein and *A* the albumin, the mobilities of which are -5.6 and -6.6×10^{-5} $cm.^2$ $volt^{-1}$ $sec.^{-1}$ respectively. Ascending and descending patterns were essentially enantiographic.

A, bovine tonsils.

B, sheep tonsils.

and was unusually sensitive to ultraviolet light (18, 19). At least two major components are present in fraction 6.2 S; one component has essentially zero mobility and the other a mobility of about -2×10^{-5} $cm.^2$ $sec.^{-1}$ $volt^{-1}$ in μ 0.10, pH 8.6 veronal

[4] Fraction 6.2 P has been examined at pH 3 in 0.10 ionic strength sodium phosphate and sodium monochloroacetate buffers; at pH 4.2 in 0.10 ionic strength sodium acetate; and at pH 9.2 in 0.02 ionic strength sodium phosphate. In each instance a single symmetrical peak was observed. It is of interest that at pH 3 the ascending and descending boundaries were not enantiographic. In the phosphate system the ascending boundary was much sharper than the descending pattern seen in Fig. 4 B; in monochloroacetate the reverse obtained. At pH 4.2 in sodium acetate the two patterns were enantiographic.

buffer. Some material precipitated when an aqueous solution of 6.2 S was allowed to stand overnight at room temperature.

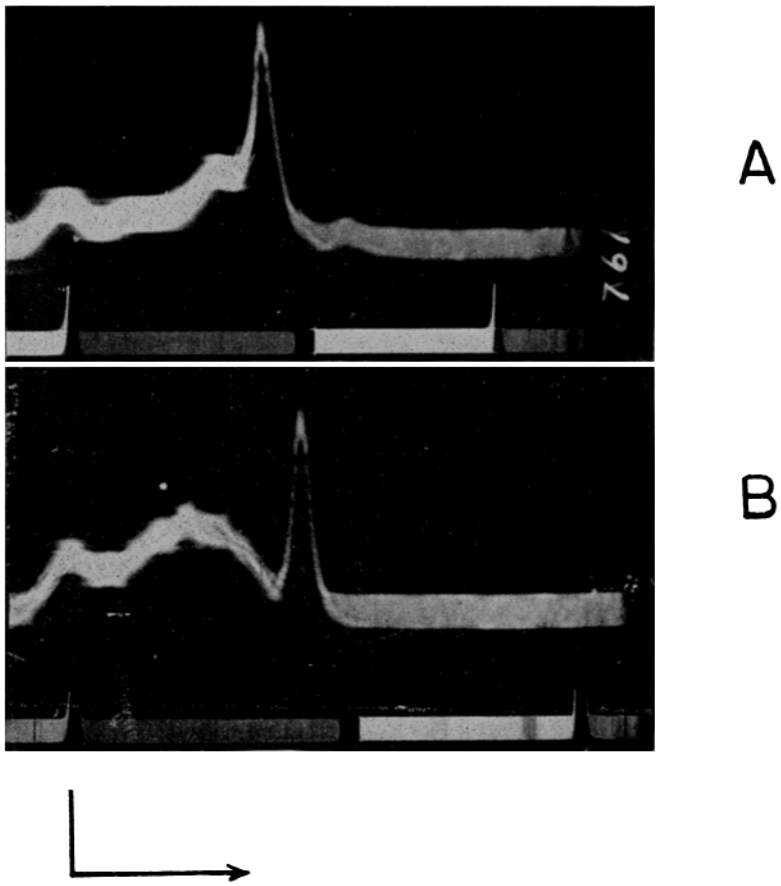

FIG. 6. Electrophoretic patterns (descending limb) of fractions 4.0 P and 4.0 S from bovine tonsils after 120 minutes under a potential gradient of 6.4 volt $cm.^{-1}$ in veronal buffer pH 8.6, $\mu 0.10$. The major component in 4.0 P is the mucoprotein, while in 4.0 S the sharp leading component is serum albumin. The mobility of the mucoprotein and albumin peaks are -5.6 and -6.6×10^{-5} $cm.^2$ $volt^{-1}$ $sec.^{-1}$ respectively. Ascending patterns closely resembled the patterns shown here.

A, pattern given by 4.0 P protein concentration about 1 per cent; diagonal slit angle 35°.

B, pattern given by 4.0 S protein concentration approximately 1.2 per cent; diagonal slit angle 40°.

Step 4 (tonsils).—The chief difference between BT, ST, and BTh was found in fraction 5.1 S and can be seen in Fig. 1 C and in Fig. 5. A component labelled *M* in Fig. 5 A, has been isolated and shown to be a mucoprotein (13). The implications of

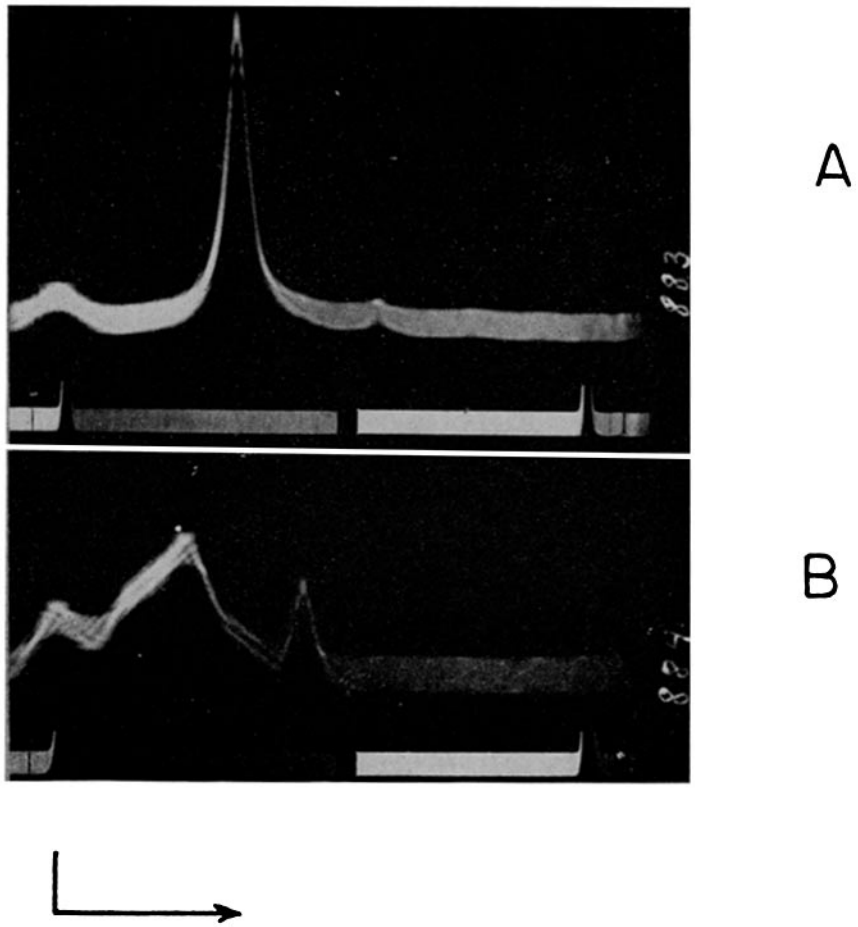

FIG. 7. Electrophoretic patterns (descending limb) of bovine thymus fractions after 120 minutes under a potential gradient of 6.4 volt cm.$^{-1}$ in veronal buffer pH 8.6, μ0.10. Ascending patterns are enantiographs of those shown.

A, fraction 4.7 P protein concentration about 1.2 per cent; slit angle 35°. The mobility of the main peak was calculated to be -4.7×10^{-5} cm.^{2} volt^{-1} sec.$^{-1}$

B, fraction 4.7 S protein concentration 1.2 per cent; slit angle 50°. The mobility of the small leading peak was -6.8 while that of the highest peak was -3.5×10^{-5} cm.^{2} volt^{-1} sec.$^{-1}$ respectively.

this finding will be discussed below. The component labelled *A* in Fig. 5 has been shown chemically, physically, and serologically to be indistinguishable from serum albumin (17).

In earlier studies fraction 5.1 S was dialyzed against water for 40 hours to remove sodium chloride. After the dialysis step the pH of the solution was lowered to 4.2 (13). The presence of a proteolytic enzyme in this fraction (21), as well as indications

of the presence of a non-proteolytic hydrolytic enzyme system, suggested the desirability of avoiding dialysis of fraction 5.1 S.

Investigation by means of the turbidity technique (24) indicated that precipitation at pH 4.0 and at μ 0.025 was as efficient as the earlier precipitation step at pH 4.2, μ 0.00. More recent fractionations have therefore been carried out at the higher ionic strength. The precipitated material (4.0 P), separated from the solution by centrifugation, was dissolved in water by adjusting the pH of the system to approximately 8 with $NaHCO_3$. The solution was dialyzed against water, and lyophilized. The dried material weighed approximately 0.8 gm.

The pH of the supernatant solution was adjusted to approximately 8 with $NaHCO_3$, the solution was dialyzed 40 hours against distilled water, frozen, and lyophilized. Approximately 1 gm. of dried solids was obtained. The electrophoretic patterns given by fractions 4.0 P and 4.0 S are shown in fig. 6 A and B respectively.

Step 4 (thymus).—In the case of thymus it was found that maximum precipitation did not occur at pH 4.0, μ 0.025. This was anticipated since fraction 4.0 P is tonsillar mucoprotein, as previously discussed, as well as other components in solution with the mucoprotein. It was found, in the case of bovine thymus, that precipitation occurred at pH 4.7, μ 0.025 and this precipitate amounted to approximately 10 per cent of fraction 5.1 S. The electrophoretic pattern obtained from the precipitate may be seen in Fig. 7 A and the corresponding pattern from the supernatant solution in Fig. 7 B. The precipitate when examined in the spectrophotometer showed a maximum at 277 mμ and a minimum at 252 mμ.

DISCUSSION

The effectiveness of fractionation step 1 is clearly revealed in Fig. 1. The major component amounting to approximately 66 per cent by weight of the initial extract (Fig. 1 A) was completely precipitated (Fig. 1 B). The substances remaining in the supernatant solution (Fig. 1 C), as mentioned above, differed considerably from the precipitate in ultraviolet absorption characteristics, chemical composition, and solubility. The above differences, considered along with the electrophoretic patterns seen in Fig. 5 and Fig. 7 suggested that several chemically and physically distinct proteins, which possessed nearly identical mobilities at pH 8.6 in μ0.10 veronal buffer, were present in the initial extract.

The presence in bovine palatine tonsils of a mucoprotein absent in bovine thymus and present in only small amount in sheep palatine tonsils allowed us to draw several conclusions. First, the mucoprotein was not a constituent of the lymphocyte. Second, it was unlikely that the mucoprotein was a constituent of connective tissue, since sufficient amounts of such tissue were present in the thymus as well as tonsils for the component to have been found in the thymus extract. The presence of fairly large amounts of epithelial tissue in bovine tonsils (13) and lesser amounts in sheep tonsils, suggests that it was this tissue that contained the mucoprotein.

The absence of color when thymus extract was heated with diphenylamine

reagent (DPA) and the presence of a purple color when both sheep and bovine tonsil extracts were heated with this reagent associates the color reaction with mucoprotein. The solutions which gave a color were examined in the spectrophotometer and found to have maxima at 520 and 650 mμ. The isolated mucoprotein (13) reacted similarly when tested with DPA. In our experience and as reported by Anderson and Maclagan (25) this color reaction with DPA appears to be a rather specific test for mucoproteins.

Certain evidence indicated that fraction 5.1 P represented a complex cytoplasmic structural element. The mobility of fraction 5.1 P (Fig. 1 B) was the same as that of the major peak in fraction A (Fig. 1 A) which suggested the presence of this entity in the cytoplasm. When fraction 5.1 P was disrupted at pH 3.0 and an ionic strength of 0.10, the nucleoprotein precipitated and 6.2 P and the materials present in 6.2 S remained in the supernate. Component 6.2 P which amounted to 25 per cent of 5.1 P was insoluble under conditions where fraction 5.1 P was soluble. Both the electrophoretic and solubility properties of 6.2 S differed from those of the parent fraction 5.1 P. The isolated nucleoprotein, which amounted to 60 per cent of 5.1 P, possessed a mobility greater than that of 5.1 P, as can be seen in Fig. 1 B and 3. Another argument in support of the above interpretation follows from an examination of extract 4 (E_4) prepared from the sediment after the third extraction. The electrophoretic pattern obtained from extract 4 closely resembled the pattern from 5.1 P (Fig. 1 B). When fractionation step 2 was applied to E_4, the products obtained resembled with respect to chemical and electrophoretic properties, fractions 3.0 P and 3.0 S prepared from 5.1 P.[5] These observations are consistent with the viewpoint that 6.2 P and the nucleoprotein existed as a complex unit within the cell.

Fraction 5.1 P contained all of the nucleic acid present in the extracts (fraction A) and with respect to lipide (8.4 per cent) nucleic acid (8.9 per cent) content resembled what have been called microsomes (26, 27). As emphasized by Palade and Siekevitz (27) most of the present information about microsomes has been derived from studies on rat liver. Microsomes separated from other tissues could differ from those of liver with respect to chemical composition. Should fraction 5.1 P represent the microsome portion of the lymphocyte we have succeeded in separating the microsome into several components.

As demonstrated by Palade and Siekevitz (27) the microsomes appear to be "fragments of endoplasmic reticulum derived to a large extent from the rough surfaced parts of the network." It may be anticipated therefore that both the chemical and physical properties of the structures might vary considerably depending upon the procedures used in their isolation. It would

[5] These results were found in the course of analysis of a series of successive extracts using calf thymus. The details of this work will be published separately.

also seem reasonable to expect that when successive extractive procedures are applied to a tissue the later extracts would become progressively richer in these fragments. Based upon this supposition our observations with E_4 as described above are consistent with the work of Palade and Siekevitz.

Although the composition of fraction 5.1 P has been fairly well defined fraction 5.1 S contains a large number of components each present in rather small amounts. In the case of tonsils, fraction 5.1 S can be conveniently separated at pH 4.0, μ0.025 into a precipitate and a supernatant solution each containing a considerable number of components. With thymus the absence of mucoproteins allowed us to obtain what appeared to be a fairly homogeneous substance as a precipitate at pH 4.7. The subfractionation of 4.0 P and 4.0 S has proved to be a difficult and time-consuming task. Some progress has been achieved, however, which indicates that the subfractions contain an abundance of interesting and possibly unusual proteins.

All the fractions discussed in this report, with the exception of fractions 5.1 P and 3.0 P, could be redissolved in water after being dried from the frozen state. Fractions 5.1 P and 3.0 P were almost completely insoluble after being frozen and/or lyophilized.

SUMMARY

A method for chemically fractionating lymphatic organs has been described. The method has been shown to be applicable to bovine palatine tonsils, sheep palatine tonsils, and bovine thymus. Approximately 50 per cent of the dry weight of tonsils and about 30 per cent of thymus has been found to be soluble in the 0.15 M NaCl extract. Four components have been isolated which together account for 65 per cent by weight of the material in the extracts. Four other components have been identified and partially defined by means of electrophoretic mobility, solubility, or some other chemical or physical property.

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BIBLIOGRAPHY

1. Halliburton, W. D., *Brit. Assn. Adv. Sc. Rep.*, 1887, **57,** 145.
2. Lilienfeld, L., *Z. physiol. Chem.*, 1893, **18,** 473.
3. Bang, I., *Beitr. chem. Physiol. u. Path.*, 1904, **5,** 317.
4. Carter, R. O., and Hall, J., *J. Am. Chem. Soc.*, 1940, **62,** 1194.
5. Mirsky, A. E., and Pollister, A. W., *J. Gen. Physiol.*, 1946, **30,** 117.
6. Carter, R. O., *J. Am. Chem. Soc.*, 1941, **63,** 1960.
7. Hall, J. L., *J. Am. Chem. Soc.*, 1941, **63,** 794.
8. Butler, J. A. V., Davison, P. F., James, D. W. F., and Shooter, K. V., *Biochem. J.*, 1954, **57,** p. XXIV.

9. Bakay, B., Kolb, J. J., and Toennies, G., *Arch. Biochem. and Biophysics*, 1955, **58,** 144.
10. Abrams, A., and Cohen, P. P., *J. Biol. Chem.*, 1949, **177,** 439.
11. Roberts, S., and White, A., *J. Biol. Chem.*, 1949, **178,** 151.
12. Harris, T. N., Moore, D. H., and Farber, M., *J. Biol. Chem.*, 1949, **179,** 369.
13. Hess, E. L., Ayala, W., and Herranen, A., *J. Am. Chem. Soc.*, 1952, **74,** 5410.
14. Peterman, M. L., Hamilton, M. G., and Mizen, N. A., *Cancer Research*, 1954, **14,** 360.
15. White, A., and Dougherty, T. F., *Endocrinology*, 1945, **36,** 207.
16. White, A., and Dougherty, T. F., *Ann. New York Acad. Sc.*, 1946, **46,** 859.
17. Hess, E. L., Campbell, M., and Herranen, A., *J. Am. Chem. Soc.*, 1954, **76,** 4035.
18. Hess, E. L., Yasnoff, D. S., and Lagg, S., *J. Am. Chem. Soc.*, 1956, **78,** 3661.
19. Hess, E. L., Yasnoff, D. S., and Lagg, S., *Arch. Biochem. and Biophysics*, 1955, **57,** 323.
20. Hess, E. L., and Lagg, S., Paper presented before The Division of Biological Chemistry, 128th National Meeting American Chemical Society, Minneapolis, September 16, 1955.
21. Hess, E. L., Campbell, M., and Herranen, A., *J. Biol. Chem.*, 1955, **215,** 163.
22. Ayala, W., Moore, L. V., and Hess, E. L., *J. Clin. Inv.*, 1951, **30,** 781.
23. Dische, Z., *Mikrochemie*, 1930, **8,** 4.
24. Hess, E. L., and Yasnoff, D. S., *J. Am. Chem. Soc.*, 1954, **76,** 931.
25. Anderson, A. J., and Maclagan, N. F., *Biochem. J.*, 1955, **59,** 638.
26. Barnum, C. P., and Huseby, R. A., *Arch. Biochem.* 1948, **19,** 17.
27. Palade, G. E., and Siekevitz, P., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 171.