tion on Sephadex so high in salt content? Since molecular size is the principal factor which determines separations on Sephadex, one would have expected the polypeptide-containing zone to be relatively free of small ions. Second, why did the CMC fail to remove as much Na and K as would be expected from its rated capacity for these cations? Desalting with glacial acetic acid was also relatively less effective. We do not know the explanation for this peculiar behavior of the material, but it may be related to its high viscosity which might interfere with contact of the extract with the particles of the exchanger. Another possibility is that other material in the LH-releasing zone may have a high positive charge which saturates the exchange capacity of CMC, and consequently interferes with retention of salts and the releasing factor.

Summary. Acetone powders prepared from sheep SME fragments were extracted with acetic acid and subjected to gel filtration on Sephadex G-25. An LH-releasing zone was eluted from the column just before the appearance of the pressor zone. The LH-releasing zones from 3 such columns were pooled, applied to a CMC column, and eluted by application of gradients of ammonium acetate of increasing ionic strength and pH. A pressor free LH-releasing zone was eluted from the CMC just prior to emergence of vasopressin. This highly purified LH-RF was active at a dose of 3 μg of peptide. Preliminary desalting was required in order for the LH-RF to be retained on the CMC column. Various steps and stages of desalting have been discussed.

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Received December 9, 1964. P.S.E.B.M., 1965, v118.

Purification and Partial Characterization of Mouse Hemopexin (Beta 2-III Globulin).* (30029)

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A protein migrating in the beta-2 globulin region has been shown to be increased in the sera of tumor bearing mice(1,2,3,4), and to be present in soluble extracts of a mouse mammary carcinoma of the RIII mice(4), and in soluble extracts of a rhabdomyosarcoma, and mammary carcinoma (both spon-

* The work was supported in part by the Adolf Friedmann Endowment, and by grants from the Junior Kielcer Aid Society, Chicago.

† This report is part of the Doctoral Thesis of I.W.

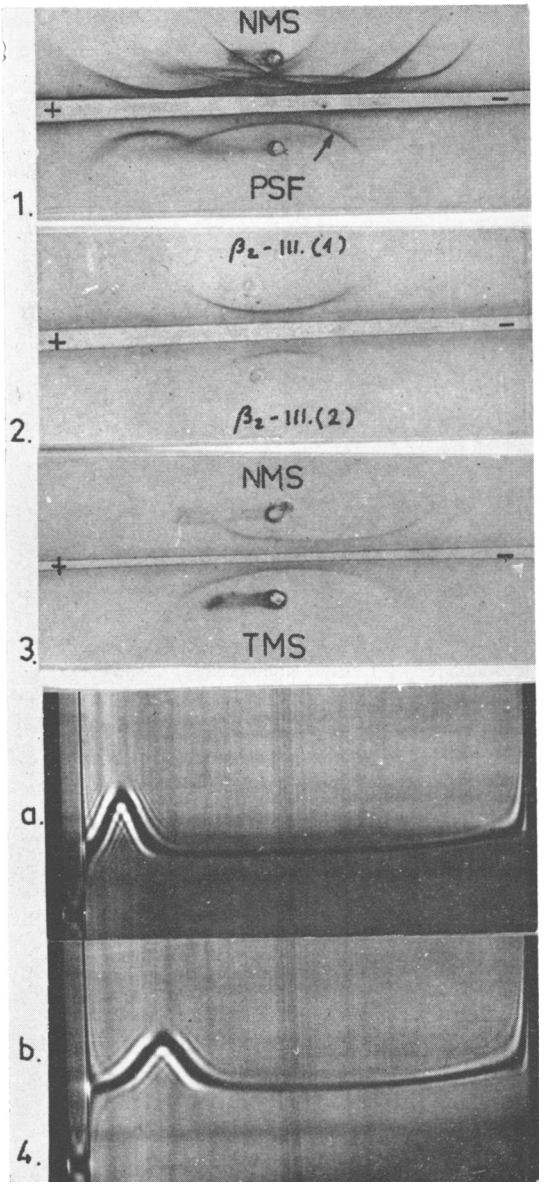

FIG. 1. Immunoelectrophoresis of normal serum (NMS) as compared to the perchloric acid soluble fraction (PSF) of mouse serum. The trough contains an antiserum to whole mouse serum. The perchloric acid soluble fraction is characterized by 2 precipitation arcs, one of which is produced by Beta 2-III globulin (arrow).

FIG. 2. Immunoelectrophoresis of 2 different preparations of purified Beta 2-III globulin. The trough contains antiserum to whole mouse serum. Note presence of a single precipitation arc.

FIG. 3. Immunoelectrophoresis of normal mouse serum (NMS) and serum from tumor-bearing mice (TMS) reacting with antiserum to Beta 2-III globulin. Note appearance of a single arc produced by the specific antiserum.

taneous and transplantable) in C3H mice (unpublished results). Initial attempts at purification resulted in a protein fraction containing 2 proteins which in immunoelectrophoresis corresponded to the Beta 2-I and Beta 2-III globulins, using the nomenclature of Clausen(2). These proteins were shown to be transferrin and a hemoglobin-binding protein, respectively(4).

Various attempts at separating these two proteins using the common methods of separation were unsuccessful. However starting with whole serum, it was possible to obtain a preparation of Beta 2-III globulin by a procedure based on perchloric acid precipitation(5) followed by rivanol (diamino ethoxyacridine lactate) treatment of the perchloric acid soluble fraction.

Materials and methods. Detailed procedure of Beta 2-III globulin purification. The procedure was carried out at 4°C and is as follows. To one volume of serum were added 9 volumes of double distilled water and 5 volumes of 1.8 N $HClO_4$. After mixing, the suspension was centrifuged at 7000 × *g* for 15 minutes. The supernate was found by immunoelectrophoretic analysis (I.E.A.), to contain the Beta 2-III globulin, plus some proteins with a greater migration to the anode (Fig. 1). The precipitate was discarded. The supernate was neutralized to pH 7.0 with approximately 5 volumes of 1.8 N NaOH. The perchloric acid treatment and neutralization was carried out in less than 30 minutes. The neutral solution was now dialysed against double distilled water for 24 hours at 4°C. The protein solution was concentrated to the original serum volume by pervaporation, at room temperature. Three volumes of a 0.4% aqueous solution of rivanol was added and the pH adjusted to pH 8.0, using 0.1 N NaOH. After mixing, the suspension was left overnight at 4°C. It was then centrifuged at 5000 × *g* for 15 minutes. The precipitate was discarded. The supernatant was concentrated to the original serum volume by pervaporation and dialysed against

FIG. 4. Ultracentrifugal analysis of mouse Beta 2-III globulin. Direction of sedimentation is from left to right. (a) after 24 min, (b) after 48 min. Sedimentation constant is 4.3 S corresponding to a molecular weight of 65000.

TABLE I. Comparison of Mouse Beta 2-III Globulin, Human Haptoglobin and Hemopexin.

	Mouse Beta 2-III globulin	Human haptoglobin (1-1)	Human hemopexin
Globulin group (pH 8.2-8.6)	Beta 2	Alpha 2 (10)	Beta 1 (11)
Hemoglobin binding capacity	+	+ (12)	+ (9)
Hematin " "	+		+ (9, 13)
Sedimentation constant	4.3	4.1 (14)	
		4.4 (15)	4.8 (14)
.4% rivanol solubility	Soluble	Not soluble (16)	Soluble (16)
.6 N $HClO_4$ "	"	Soluble (17)	" (11)
Hexose %	7.2	7.8 (14)	
		7.7-10.4 (15)	9.0 (14)
Hexosamine %	6.2	5.3 (14)	
		5.6 (15)	7.4 (14)
Sialic acid %	4.0	5.3 (14)	
		6.3 (15)	5.8 (14)

0.9% sodium chloride for 16 hours at 4°C. The resultant protein solution showed the presence of a single precipitation arc (Fig. 2) when examined by I.E.A. The protein content of the solution was approximately 1% of the total serum proteins.

Procedures for immunoelectrophoresis protein determination, hemoglobin binding and antisera preparation were as described previously(4).

Ultracentrifugal analysis was performed in a Spinco Model E ultracentrifuge at 56,000 rpm and a temperature of 17°C.

Glycoprotein staining was performed according to Uriel and Grabar(6). Protein bound Hexose and Hexosamine determination were performed as described by Winzler(7) and sialic acid by the method of Warren(8). Hematin-binding was determined according to Neale *et al*(9).

Results and discussion. The antigenic purity of the protein preparation was examined by preparing rabbit antisera against it. In I.E.A. all such specific antisera reacted with the purified protein to give a single precipitation arc. However when tested against whole serum, 2 additional faint precipitation arcs could be detected. The antibodies producing those lines were no longer detectable when the rabbits were bled 2 months after the last immunization (Fig. 3). This would indicate the presence of very small amounts of 2 antigenic contaminants in the immunizing preparation.

Ultracentrifugal analysis of the protein revealed the presence of a single peak (Fig. 4). The sedimentation constant was S20 = 4.33×10^{-13}, from which the molecular weight could be calculated to be approximately 65,000.

The precipitation arc of Beta 2-III globulin was found to stain for glycoprotein. Chemical analysis of the pure fraction confirmed this characteristic and showed that the protein contained 7.2% of Hexose (glactose mannose—determinations of 2 different preparations gave 7.0% and 7.3%), 6.2% of Hexosamine (three different preparations had values of 6.0%, 6.2% and 6.4%) and 4% of sialic acid (three different preparations gave identical results—4%).

The globulin retained its hemoglobin binding capacity and in addition was found to bind hematin.

The characteristics of this mouse Beta 2-III globulin can now be summarized and compared with the 2 analogous hemoglobin-binding proteins of human serum (Table I).

It is clear that this protein is a glycoprotein of the "seromucoid" group(7) resembling the Beta 1 hemopexin of human serum (11) and differing from human Alpha 2 haptoglobin primarily in its hemin binding capacity, its mobility, and its rivanol solubility. It should be noted, however, that human hemopexin appears in the perchloric acid soluble fraction only when the initial dilution of serum is done with saline. When, however, human serum is initially diluted with twice distilled water, as performed in the present work, hemopexin is not demonstrable in the perchloric acid soluble fraction. It is suggested that the names Beta 2-III globulin, and mouse haptoglobin(18) be replaced by

the term mouse hemopexin to identify this protein constituent of mouse serum.

Summary. A method for purification of mouse Beta 2-III globulin is described. The purified protein is perchloric acid soluble and contains hexose, hexosamine and sialic acid. It is soluble in rivanol and binds hematin. It has a sedimentation constant of 4.3 S, indicating a molecular weight of about 65,000. It is suggested that this protein henceforth be called mouse hemopexin.

The technical assistance of Miss C. Brunschwig is acknowledged with appreciation. The authors also wish to thank Mr. P. Yanai for ultracentrifugal analysis, Dr. E. R. Berman for hexosamine analysis and Mrs. J. Naaman for sialic acid determinations.

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Received December 11, 1964. P.S.E.B.M., 1965, v118.

Sensitivity of Bluetongue Virus to Ether and Sodium Deoxycholate.* (30030)

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Arboviruses as they are presently defined, in addition to biologic transmission by arthropod vectors, have other properties in common. All are believed to contain RNA as their genetic material and all so far examined are inactivated by treatment with ether or sodium deoxycholate (SDC). Evidence that bluetongue virus is transmitted by arthropod vectors is compelling(1-3). Evidence that this transmission is biologic in the sense that the virus infects and multiplies within the cells of the insect before it is transmissible to susceptible sheep, while convincing (3), is not definitive. The genome of bluetongue virus is believed to be RNA(4). Andrewes(5) has indicated that bluetongue virus is not inactivated by treatment with ether or SDC. In the present study the sensitivity of 2 strains of bluetongue virus to ether or SDC was examined.

Materials and methods. A strain of bluetongue virus, BT-Cy, recovered from infected sheep in Cyprus(6) and a strain, BT-8, recovered from infected sheep in California(7) were examined for sensitivity to ether or SDC. Both viruses were originally isolated and passaged by yolk sac inoculation of chicken embryos (CE). BT-Cy at the 82nd CE passage and BT-8 at the 78th CE pas-

* This investigation was supported in part by grants from U.S.P.H.S. and U. S. Department of Agriculture.