stant with an aortic clamp. It was demonstrated that the drug increased sodium excretion in the absence of blood flow alterations. The increase was less than that seen without the clamp. These data are interpreted as indicating that this agent acts in part by directly inhibiting sodium reabsorption and in part by altering renal hemodynamics to increase sodium excretion.

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Differences in Electrophoretic Migration of Mouse $7S\gamma_{2a}$ and $7S\gamma_{2b}$ Globulins (32656)

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The mouse immunoglobulin system consists of four major classes of globulins—$7S\gamma_1$, $7S\gamma_2$, γ_{1A}, and γ_{1M} (1) and the $7S\gamma_2$ class has two antigenically distinct subclasses, the $7S\gamma_{2a}$ and $7S\gamma_{2b}$ globulins (2). The $7S\gamma_2$ subclasses are of considerable interest because of differences in their isoantigens (3–6) and biologic functions (7). However, studies with these two subclasses have been limited to the use of $7S\gamma_{2a}$ and $7S\gamma_{2b}$ myeloma proteins because a technique for separating normal $7S\gamma_{2a}$ and $7S\gamma_{2b}$ globulins has not been described. This report describes differences in the electrophoretic migration of $7S\gamma_{2a}$ and $7S\gamma_{2b}$ globulins of various inbred strains of mice and shows that partial separation of these two proteins may be obtained by electrophoretic methods.

Materials and Methods. Inbred mice and mouse serums were obtained from Roscoe B. Jackson Memorial Laboratories, Bar Harbor, Maine. Noninbred mouse serums were secured from Colorado Serum Company (CSC), Denver, Colorado and from RML mice maintained at the Rocky Mountain Laboratory.

Hen albumin (HEA) was obtained from K and K Laboratories, Inc., Jamaica, New York and iodinated with I^{131} (E. R. Squibb and Sons, Inc., New Brunswick, New Jersey), as previously described (8). Myeloma proteins LPC-1, MPC-31, and MPC-47 representing the $7S\gamma_{2a}$, $7S\gamma_{2b}$, and $7S\gamma_1$ groups, respectively, were obtained from Dr. J. L. Fahey and Dr. R. Asofsky (NIH, Bethesda, Maryland).

New Zealand white rabbits and Targhee sheep from local sources were used for the production of antiserums. Rabbit antiserums to Balb/c $7S\gamma_1$ globulins and mouse antiserums to HEA were prepared as previously described (9). Antiserums to $7S\gamma_{2a}$ and $7S\gamma_{2b}$ globulins were prepared in rabbits by injection of the respective myeloma protein in complete Freund's adjuvant. Specificity was achieved by absorption with the heterologous $7S\gamma_2$ myeloma protein and verified by gel-diffusion analysis with $7S\gamma_{2a}$, $7S\gamma_{2b}$, and $7S\gamma_1$ myeloma proteins. Rabbit and sheep antiserums to mouse serum were prepared by injecting whole mouse serum emulsified in complete Freund's adjuvant and then 3–4 weeks later starting weekly injections of the antigen in incomplete

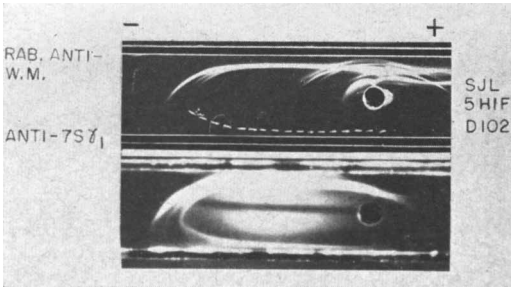

FIG. 1. Immunoelectrophoresis pattern (top) and corresponding HEA ^{131}I autoradiograph (bottom) of SJL serum obtained 102 days after inoculation with 5 μg HEA in incomplete Freund's adjuvant. Rabbit antiserum (top trough) prepared against whole mouse serum (Colorado Serum Company) forms three precipitin lines in the gamma region and the most anodal precipitin line fuses with the precipitin line formed by the specific $7S\gamma_1$ antiserum. A dashed white line was added to identify the faint $7S\gamma_1$ precipitin line.

adjuvant. Antiserums of suitable quality were usually obtained after 3–6 booster injections.

Gel diffusion, immunoelectrophoresis, and autoradiography tests were performed as previously described (9) except that the immunoelectrophoresis slides were routinely washed with saline and photographed prior to the addition of radioactive antigen for autoradiography purposes. Pevikon-block electrophoresis with Pevikon from Fosfatbolaget, Stockholm 5, Sweden, was performed according to Muller-Eberhard (10) with a barbital buffer of pH 8.6 and ionic strength of 0.05. Electrophoresis was done at 4°C for 21–34 hours and then 1-cm sections of the block were eluted with saline in a centrifugal elution device.

Results. Immunoelectrophoretic examination of SJL mouse serum revealed three distinct precipitin lines in the gamma region (Fig. 1, top) when a rabbit anti-CSC mouse antiserum was used to precipitate the mouse serum proteins. All three precipitin lines represented immunoglobulins as shown by the specific binding of HEA ^{131}I when mouse serums containing HEA antibody were tested by radioimmunoelectrophoresis (Fig. 1, bottom). The most anodal of the three gamma globulin precipitin lines represented the $7S\gamma_1$ globulin as shown by the reaction with the precipitin line formed by the rabbit $7S\gamma_1$ antiserum (Fig. 1, bottom trough). The immunoelectrophoresis pattern in Fig. 2 shows the relationship of the $7S\gamma_{2a}$ and $7S\gamma_{2b}$ precipitin lines to the three gamma precipitin lines which were obtained with a sheep antiserum against RML mouse serum. As in Fig. 1, the most anodal precipitin spur (arrow) merged with the precipitin line formed by $7S\gamma_1$ antiserum (not shown). The most cathodal precipitin spur represented the $7S\gamma_{2a}$ globulin as shown by the reaction with the precipitin line formed by specific $7S\gamma_{2a}$ antiserum (Fig. 2, top trough). The $7S\gamma_{2b}$ antiserum (Fig. 2, bottom trough) formed a precipitin line which was slightly more anodal in position than the $7S\gamma_{2a}$ precipitin line, but did not clearly merge with the intermediate gamma-globulin spur. The identity of the intermediate gamma-globulin line was established by the arranagement shown in Fig. 3. Coincident with charging the trough after completion of electrophoresis, a second well was made at an appropriate site and filled with $7S\gamma_{2b}$ antiserum. The precipitin line formed by the anti-$7S_{2b}$ from this well merged with the intermediate gamma

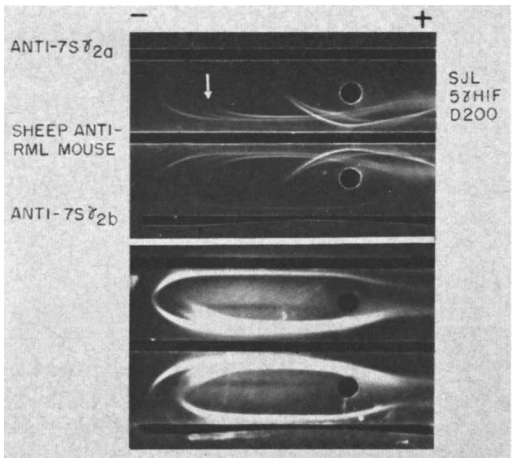

FIG. 2. Immunoelectrophoresis pattern (top) and corresponding HEA ^{131}I autoradiograph (bottom) of immune SJL serum (in wells) when reacted with specific $7S\gamma_{2a}$ antiserum (top trough) and specific $7S\gamma_{2b}$ antiserum (bottom trough). The most anodal precipitin line in the gamma region (arrow) represents $7S\gamma_1$ globulin and the most cathodal precipitin line merges with the $7S\gamma_{2a}$ precipitin line. The $7S\gamma_{2b}$ appears slightly faster than the $7S\gamma_{2a}$. A pattern similar to this was obtained with $C_{57}BL/6$ serum in the wells.

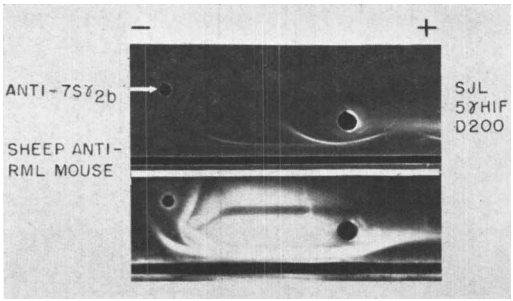

FIG. 3. Immunoelectrophoresis pattern (top) and corresponding HEA ^{131}I autoradiograph (bottom) of SJL serum containing HEA antibody. Antiserum to $7S\gamma_{2b}$ was placed in the well made after electrophoresis, coincident with charging the trough with sheep antiserum. The specific $7S\gamma_{2b}$ precipitin line fuses with the intermediate precipitin line formed in the gamma region by the sheep antiserum.

precipitin spur and was crossed by the $7S\gamma_1$ precipitin line (see autoradiograph, Fig. 3). Therefore the intermediate precipitin line in the gamma region produced by the sheep anti-RML serum, represented a reaction with the $7S\gamma_{2b}$ globulin. The slowest and intermediate precipitin lines produced by the antiserum used in Fig. 1 were similarly identified as $7S\gamma_{2a}$ and $7S\gamma_{2b}$ globulins, respectively. In addition to the rabbit anti-CSC mouse serum (Fig. 1), and sheep anti-RML mouse serum (Figs. 2, 3, 4), a sheep anti-CBA mouse serum and a rabbit anti-RML mouse serum also precipitated three separate gamma-globulin precipitin lines on immunoelectrophoresis. The 7S gamma-precipitin lines produced by these antiserums were analogous in that the most cathodal line represented the $7S\gamma_{2a}$, the intermediate line the $7S\gamma_{2b}$ and the most anodal line the $7S\gamma_1$ globulin. A similar relationship of the three 7S gamma globulins was seen by immunoelectrophoretic examination of serums obtained from SJL, $C_{57}BL/6$, CBA, Balb/c, C_{58}, and C_3H strains but was most easily visualized using SJL or $C_{57}BL/6$ serum. Normal and immune SJL serums had similar patterns, but the relationships of the 7S globulins were demonstrated most clearly by using HEA ^{131}I radioimmunoelectrophoresis tests of SJL serums which contained HEA antibody. [Inoculation of SJL mice with small amounts of HEA in incomplete or complete adjuvant induces the prompt appearance of antibody in the $7S\gamma_1$, $7S\gamma_{2a}$, and $7S\gamma_{2b}$ globulins in contrast to observations with CBA and Balb/c mice (9)]. The $7S\gamma_{2b}$ of CBA or Balb/c serum did not appear to migrate as slowly as that of SJL, and on immunoelectrophoresis, the $7S\gamma_{2b}$ precipitin line was closer to the $7S\gamma_1$ precipitin line (Fig. 4). Previous studies have shown that the $7S\gamma_1$ of CBA or Balb/c serum does not migrate as slowly as that of SJL or $C_{57}BL/6$ serum (11).

To confirm the difference in electrophoretic mobility of the $7S\gamma_{2a}$ and $7S\gamma_{2b}$ globulins, normal SJL, $C_{57}BL/6$, CBA, or Balb/c serums were separated by Pevikon-block preparative electrophoresis and the fractions tested for the presence of the $7S\gamma_2$ subclasses by using specific $7S\gamma_{2a}$ and $7S\gamma_{2b}$ antiserums in gel-diffusion tests (Table I). The slowest (most cathodal) globulin consistently represented $7S\gamma_{2a}$ globulin without detectable $7S\gamma_{2b}$ globulin. The Pevikon-block separation of CBA and Balb/c serums showed that $7S\gamma_{2a}$ was present throughout those fractions which contained $7S\gamma_{2b}$ globulin whereas with SJL or $C_{57}BL/6$ serums some $7S\gamma_{2b}$ could be detected in a location anodal to the $7S\gamma_{2a}$ migration.

Discussion. Studies on the allotypic specificities and biologic functions of the $7S\gamma_{2a}$ and $7S\gamma_{2b}$ globulins of the mouse have heretofore been restricted to the use of myeloma proteins. The use of normal $7S\gamma_2$ globulins and of antibody containing $7S\gamma_2$ globulins for these studies would be of obvious importance. Myeloma proteins represent a restricted mono-

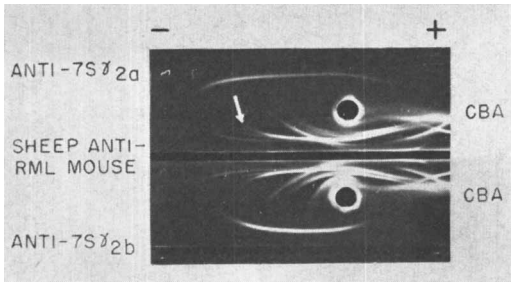

FIG. 4. Immunoelectrophoresis pattern of CBA serum (in wells) with whole mouse antiserum in center trough and specific $7S\gamma_{2a}$ and specific $7S\gamma_{2b}$ antiserums in top and bottom troughs, respectively. The $7S\gamma_1$ line (arrow) was identified with specific $7S\gamma_1$ antiserum (not shown). A pattern similar to this was seen with Balb/c serum in the wells.

TABLE I. Separation of Mouse $7S_{\gamma 2a}$ and $7S_{\gamma 2b}$ by Pevikon-Block Electrophoresis.

Strain of mouse	Duration of electrophoresis (hours)	Type of $7S_{\gamma 2}$	Pevikon block fraction																			
			6	7	8	9	10[a]	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
SJL	34	a	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0
		b	0	0	0	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0
$C_{57}BL/6$	21	a	0	0	0	+	+	+	+	+	+	+	+	0	0	0	0							
		b	0	0	0	0	+	+	+	+	+	+	+	+	0	0	0							
CBA	21	a	0	0	0	+	+	+	+	+	+	+	+	+	+	+	+	+	0					
		b	0	0	0	0	0	+	+	+	+	+	+	+	0	0	0	0	0					
Balb	23	a	0	0	0	0	+	+	+	+	+	+	+	+	+	+	+	0						
		b	0	0	0	0	0	+	+	+	+	+	+	+	+	0	0	0						

[a] Origin.

clonal, sometimes atypical immunoglobulin (6) which usually has no detectable antibody activity.

The ability of two $7S_{\gamma 2a}$ and two $7S_{\gamma 2b}$ myeloma proteins to fix to heterologous skin was tested by reverse PCA (7). Only the $7S_{\gamma 2a}$ myeloma proteins were found to have this biologic function. Thus in the mouse, where the $7S_{\gamma 2a}$ is electrophoretically slower than the $7S_{\gamma 2b}$, the slowest of the $7S_{\gamma 2}$ globulins has heterologous skin-fixing ability. This corresponds to studies in other animals where the slowly migrating 7S gamma globulin was shown to mediate heterologous skin sensitization (12).

In all four strains tested, $7S_{\gamma 2a}$ globulin free of detectable $7S_{\gamma 2b}$ globulin could be obtained by Pevikon-block electrophoresis. Previous studies provide additional evidence that $7S_{\gamma 2a}$ can be separated from $7S_{\gamma 2b}$ globulin by this technique. Inoculation of rabbits with the most cathodal $C_{57}BL/6$ globulin isolated by Pevikon-block electrophoresis induced the formation of antibody with $7S_{\gamma 2a}$ specificity (and IgI^b allotype specificity) but no antibody with $7S_{\gamma 2b}$ specificity (13). The $7S_{\gamma 2b}$ globulin without detectable $7S_{\gamma 2a}$ globulin could be obtained by Pevikon-block separation of $C_{57}BL/6$ and SJL serum, whereas such separation could not be obtained with CBA and Balb/c serums. These relationships between the $7S_{\gamma 2a}$ and $7S_{\gamma 2b}$ globulins could also be seen on the immunoelectrophoresis test and may represent quantitative or perhaps qualitative differences between the $7S_{\gamma 2a}$ and $7S_{\gamma 2b}$ globulins of inbred strains of mice.

This study shows that $7S_{\gamma 2}$ subclasses frequently can be detected by use of sheep or rabbit antiserums prepared against whole mouse serum. The separate $7S_{\gamma 2a}$ and $7S_{\gamma 2b}$ spurs are most easily discerned on immunoelectrophoresis by electrophoresing an appropriate serum (such as SJL) for a prolonged time (90–120 min 7 V/cm) and amplifying the weak precipitin lines by autoradiography. Recent studies have shown that agarose is a superior milieu for the immunoelectrophoretic demonstration of differences in migration between the 7S globulins when compared to each other and when compared to those of another strain of mouse.

Conclusion. The $7S\gamma_{2a}$ and $7S\gamma_{2b}$ globulins of mice differ in their electrophoretic migration. The $7S\gamma_{2a}$ globulin was seen to be slower electrophoretically than $7S\gamma_{2b}$ globulin in the four inbred strains of mice examined and $7S\gamma_{2a}$ globulin free of $7S\gamma_{2b}$ globulin could be obtained by Pevikon-block electrophoresis. Antiserums prepared in rabbits and sheep by inoculation with whole mouse serum frequently contain antibody specific for the $7S\gamma_{2a}$ and $7S\gamma_{2b}$ globulins as well as the $7S\gamma_1$ globulin, and these specificities can be detected on routine immunoelectrophoresis.

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Effects of Antilymphocyte Serum on Virus Oncogenesis (32657)

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It is generally accepted that virus-induced tumors have antigens demonstrable by transplantation resistance or other methods (1,2). Much remains to be learned however, about the role of immune reactions in controlling tumor growth. Experiments with immunosuppressive procedures are helpful in the analysis of this problem. In general, chemical immunosuppressive agents have limited application because they may also affect virus or tumor cell multiplication, and this can make interpretation of results difficult. Comparisons of tumor incidence in neonatally thymectomized and intact animals exposed to viruses or chemical carcinogens have already provided much useful information (3–5). Mice or rats infected as newborns or later with polyoma virus, simian virus 40 or adenovirus type 12 consistently show more tumors after neonatal thymectomy than intact animals infected at the same time. Animals treated with chemical carcinogens sometimes show increased incidence of tumors after neonatal thymectomy, but usually do not. Mice infected as newborns with the mammary tumor agent or Passage A (Gross) or MLV (Moloney) leukemogenic viruses develop fewer mammary tumors or leukemias after neonatal thymectomy than do intact animals infected with these agents (3, 6–8).

Inhibition of leukemogenesis cannot be attributed merely to the removal of thymic cells sensitive to neoplastic conversion. Law and Potter (9) and Carnes *et al.* (10) found that in thymectomized animals restored by genetically identifiable thymus grafts the leukemias which developed were often of host origin and did not originate from the cells or donor thymic tissue. These findings suggest that nonlymphoid elements of the thymus (epithelial and reticular cells) facilitate the conversion of normal to leukemic cells within the thymus. Nevertheless the effect of thymectomy is clearly something other than immunosuppression, and analysis of the role of immunity in virus leukemogenesis calls for the application of a different immunosuppressive procedure.