mide on intravenous toxicity in mice, on *in vitro* mast cell disruption, and on antiheparin potency. Polymers of average molecular weight as well as dialysates of several such polymers were used in these studies. Results showed (a) that there is a definite trend for the toxicity of hexadimethrine to increase concomitantly with an increase in average polymer size, (b) that both intravenous toxicity and effects on mast cells are interrelated through the average molecular size of the samples used, and (c) that dialysis can fractionate the average molecular weight polymers into their components of varying toxicity without appreciable loss of antiheparin potency.

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Suppression of Antibody Forming Capacity with Thymectomy in the Mouse.* (27699)

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Previous reports have indicated that surgical ablation of the thymus gland has a variable effect on the immune response of the operated animal. While the immunologic-depressive effect of thymectomy appeared to be a function of the age at which the thymus was removed(1-5), further investigation has also revealed wide difference in immunologic maturation at birth or hatching in various species(6-9). Thymectomy within 24 hours after birth in the rabbit appeared to lower the response to antigenic stimulation when antibody was measured by quantitative technics(10). However, thymectomy, even within the immediate neonatal period, did not completely abolish the immune response. We report here that complete thymectomy performed within 24 hours after birth in the DBA/2 strain of mice almost completely abolishes the ability of survivors to respond to stimulation when bacteriophage T_2 (*E. coli* B) is used as antigen.

Material and methods. Newborn mice of the DBA/2 strain were thymectomized by a procedure described previously(5). Sham operated controls were subjected to a similar operative procedure, but the thymus was left intact. At 2 months of age, controls and thymectomized survivors were given a single dose of T_2 bacteriophage (2×10^{10} particles) intraperitoneally and bled 7 days later from the retro-orbital plexus into heparinized capillary tubes. The tubes were centrifuged at 4°C at $1500 \times g$ and the serum removed. Virus

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TABLE I. Phage Neutralization in Antiserum from Normal and Thymectomized Mice.

Animal No.	% neutralization at 2 hr	% neutralization at 24 hr	Amt of thymus at autopsy*
Diluent control (20% NRS-saline)	5	60	
Sham operated control group			
51	80	Complete	Intact
52	69	"	"
53	68	"	"
54	86	"	"
55	63	"	"
56	50	"	"
57	10	"	"
Thymectomized group			
1	49	Complete†	1/3 present
2	3	70	Absent
3	6	66	"
10	10	92	Trace present
12	5	60	Absent
14	30	60	"
15	10	72	"
17	31	93	Trace present

* On the basis of gross examination of tissues.
† Below the limit of detection at a 1:1000 dilution from reaction tube.

neutralization measurements were performed at 37°C with serums from control and thymectomized animals diluted 1:50 in normal rabbit serum (20% serum in 0.9% NaCl). Incubations were carried out over 24 hours with an initial concentration of 10^5 virus particles in the reaction tubes.

Results. A clear cut difference was noted in the phage neutralizing power of antiserums obtained from the thymectomized and control mice. After 2 hours' incubation there was an obvious difference in neutralization rate between the 2 groups except for one of the thymectomized mice (Table I). After 24 hours of incubation, neutralization of virus was complete in the serum from control animals, but only one from the thymectomized group showed complete neutralization at this time.

At autopsy, all the sham operated animals had an intact thymus. Animals in the thymectomized group showing complete absence of thymic tissue as evaluated in the gross were those numbered 2, 3, 12, 14 and 15 in Table I. Mouse No. 1 possessed approximately 1/3 of a normal thymus, while smaller traces of residual thymus remained in Mice 10 and 17.

Discussion. These results demonstrate that antibody forming capacity can be almost completely abolished by prior thymectomy in the mouse if the operation completely removes the thymus and is performed sufficiently early in the animal's development. With the sensitive technic of antibody measurement, bacterial virus neutralization, used in this study, no inactivation attributable to antibody could be found in the sera of mice in which complete thymectomy was proved at autopsy. Such a profound depression of the immune response is consistent with other data obtained from studies employing homografting(11,12) and cell transfer studies with graft *vs* host reaction as the indicator of immunological response.

The studies reported herein indicate that all of the thymus must be removed at time of operation in order to completely eliminate the immune response. It is possible that small remnants may grow to a sufficiently large size to reconstitute immunologic capacity and return it to levels approaching those of the normal mouse.

Summary. Thymectomy performed within 24 hours after birth in the DBA/2 strain of mice almost completely abolishes the ability of these animals to respond to antigenic stimulation with T_2 bacteriophage. These and other recent studies indicate that the information necessary for development of capacity for immunologic recognition of foreign antigens is contained within the thymus of the mouse during the fetal-neonatal period.

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Polybasic Electrolytes and Colloid Osmotic Pressure of Plasma, Serum and Bovine Serum Albumin Solution.* (27700)

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Several polybasic macromolecular substances and basic dyes produce pulmonary edema in the perfused lung(1,2). There are three plausible mechanisms for this phenomenon. These substances 1) may produce arteriolar occlusion by agglutination of red cells, 2) may cause polymerization of plasma protein, thus reducing the colloid osmotic pressure, or 3) there may be chemical action on the capillary endothelium which increases permeability to protein. The first possibility has been shown to occur. Sela and Katchalski(3) have reviewed the literature relating to this phenomenon. The contributions of the other 2 mechanisms have not been established.

The purpose of this study was to investigate the effect of the basic dyes, Neutral red and Methylene blue, and the polybasic macromolecular substances, protamine (spermine · 4HCl) and Polybrene (hexadimethrine bromide) on the colloid osmotic pressure of plasma, serum, and a 6% albumin solution *in vitro*. Of the 4 substances tested, all except Neutral red were found to produce pulmonary edema by Visscher *et al.*(1).

Methods and materials. The colloid osmotic pressure of the test solutions was measured with an adaptation of a Statham strain gauge head$_2$, a 0-75 cm Hg strain gauge, and a Sanborn recorder(4). The membrane used was dialysis membrane supplied by Visking Corp. The closed lower chamber was filled with the reference buffer solution and the colloid containing solution to be tested (0.5 cc) was placed in the upper chamber of the head which was open to atmospheric pressure. The reference buffer solution was Krebs-Ringer buffer solution (0.81% NaCl, 0.041% KCl, 0.0049% $CaCl_2$, 0.019% KH_2PO_4, 0.034% $MgSO_4$, 0.15% Na_2HPO_4) pH = 7.4. The measurements were made at laboratory room temperature, approximately 22°C.

Pressure equilibrium was obtained in about 35-45 minutes with the plasma and in 20-30 minutes with the albumin solution.

The albumin solution was prepared by dissolving 0.6 g of powdered bovine serum albumin, MW = 69,000, in 10 cc of the Krebs-Ringer buffer. All of the substances to be tested were prepared in buffer solutions and in such concentrations that the plasma and albumin solutions were not diluted by more than 4% upon their addition. The final concentrations of the test substances are indicated in Table I.

Results. The effects of the various substances on the measured colloid osmotic pressures are summarized in Table I. In the concentrations employed none of the materials tested had significant effect on the C.O.P. The polybasic macromolecular substances changed the final pH of the test solutions by only 0.1 pH unit. The volume of diluent employed had a dilution effect of less than 1 mm Hg.

Conclusion. It has been shown that several materials causing pulmonary edema in isolated dog lungs under constant perfusion conditions have no significant effect on the plas-

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