as opposed to the works cited(1-3), the material employed for pretreatment was from a pooled source and not from the skin donors.

The low nucleic acid content of our material limits coordination of our findings with the hypothesis presented by Billingham *et al.* (4). Experiments are underway to evaluate the role of a polysaccharide material isolated from rat glomerular basement membrane by a method reported elsewhere(8) in enhancing takes of skin grafts.

Summary. Intraperitoneal injections of pooled rat glomerular basement membrane preparations into rats subsequently homografted with a primary orthotopic skin graft facilitated a secondary rejection phenomenon on the part of such treated animals. The low nucleic acid content of the preparations limited correlation with the hypothesis of nucleoproteins as the transplantation antigens.

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Occurrence and Enzymatic Degradation of Phosphoethanolamine.* (24491)

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The biological occurrence of phosphoethanolamine was first observed in bovine tumor tissue by Outhouse(1), who failed to find it present in normal tissues. Colowick and Cori(2) demonstrated the ester to be present in rabbit and pig intestine. Lately it has been shown to occur in a variety of normal tissues and often in quite large amounts(3,4,5,6). Thus far, the major biochemical interest of the compound lies in its possible role as an intermediary in the metabolism of phospholipids. Little is known of other possible functions, or of the enzymology of phosphoethanolamine metabolism. Roche and Bouchilloux (7) have shown that dog liver, human prostate, and beef kidney split phosphoethanolamine at pH 5.6, although at a lower rate than glycerophosphate. Strickland *et al.*(8) reported that alkaline phosphatase of various tissues readily split phosphoethanolamine, but that there was a relative inactivity toward this phosphate ester by acid phosphatase of these same tissues. This paper reports information about distribution of the compound in various tissues, and its enzymatic degradation by tissue homogenates under different conditions, *i.e.*, pH, buffer, metal ions, etc.

Methods. Phosphoethanolamine was synthesized by the method of Outhouse(9) and phosphate determinations were made by the method of Gomori(10). As soon as animals were sacrificed, samples of tissue were immediately homogenized with Teflon pestle in distilled water. Aliquots of the homogenate were treated with 10% trichloroacetic acid to remove proteins, and after removal of the acid with ether the resulting solution was concentrated *in vacuo*. Quantitative determination of phosphoethanolamine was then made by Dowex-50 chromatography as previously described(5). The incubation system contained in final volume of 11 ml, 9 ml of buffer (veronal-acetate, pH 4.5, or veronal, pH 9.0), 1 ml of substrate (15 x 10^{-5} moles of either phosphoethanolamine or beta-glycerophosphate, and 5 x 10^{-3} moles of $MgCl_2$), and 1 ml homogenate. Incubations were carried out in small Erlenmeyer flasks with shaking in

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TABLE I. Distribution of Phosphoethanolamine in Rat and Rabbit Tissues. Values expressed as mM/100 g dry tissue and probable error of the mean.

Tissue	Rat*	Rabbit†
Liver	.18 ± .01	2.72 ± .06
Kidney	.48 ± .07	2.34 ± .19
Heart	.47 ± .17	.36 ± .00
Spleen	2.24 ± .19	2.57 ± .30
Brain	.50 ± .02	.90 ± .00
Muscle	.21 ± .05	.10 ± .00
Ret. cell sarcoma	1.08 ± .06	
Walker Ca.	.88 ± .04	

* Avg of 3 animals. † Avg of 2 animals.

constant temperature bath at 37°C. After one hour, the reaction was terminated by addition of 5 ml of 20% trichloroacetic acid. Inorganic phosphorous was determined similarly except that the substrate was omitted. A 1 ml aliquot of each homogenate was removed and kept at 100°C for 24 hrs for determination of dry weight. Values are expressed as μM of P released/g dry tissue/hr.

Results. Determinations made of phosphatase activity at intervals of 0.5 pH units from 4 through 10 showed the velocity of hydrolysis of phosphoethanolamine by various tissue homogenates to have 2 maxima at pH 4.5 and 9.0. Rates of hydrolysis by various tissue homogenates were compared in borate, veronal, tris, citrate, and acetate buffers. Although differences were slight, veronal at pH 9.0 and veronal-acetate at pH 4.5 gave maximum hydrolysis of the ester. The effect on hydrolysis rate of the ester at pH 9.0 by Mg^{++} was determined on muscle, brain, and heart homogenates. The rate increased 100, 0, and 20% respectively, whereas Ca^{++} at this pH had no effect. At pH 4.5, neither ion had any effect on hydrolysis of the ester by these 3 tissues; however Mg^{++} increased hydrolysis of glycerophosphate by 50%. Under optimal conditions described, the reaction velocity using spleen and kidney homogenates at pH 9.0 remained essentially constant throughout a 120 minute period.

Table I illustrates concentration of phosphoethanolamine in 6 rat and rabbit tissues as well as 2 transplantable rat tumors. It can be seen there is a species difference, rabbit liver and kidney having considerably more of the ester than the corresponding tissues of the rat. The value for spleen in both species was large and similar results have been reported for lymphoid tissue by other authors(5,11).

Table II shows that for each tissue, glycerophosphate is hydrolysed by alkaline phosphatase at 2-6 times the rate for phosphoethanolamine. Only muscle of both species showed a lower ratio of approximately 1. The results of Table II also show that the ester of ethanolamine is hydrolysed at a considerably slower rate by acid phosphatase (pH 4.5) than glycerophosphate. It is obvious, however, that the ratio of hydrolysis of the 2 esters at the acid pH is not constant from one enzyme source to another, *viz.*, rat kidney, reticulum cell sarcoma, and Walker 256 carcinoma showed little activity toward the ester of ethanolamine; however the acid phosphatase of these 3 same tissues was quite active toward glycerophosphate. It is obvious from Table II that human prostate split glycerophosphate at 3 times the rate of phospho-

TABLE II. Hydrolysis of Phosphoethanolamine by Various Tissue Homogenates. Values expressed as μM/g dry tissue/hr.

	Rat				Rabbit			
	pH 4.5		pH 9.0		pH 4.5		pH 9.0	
Tissue	PEt	BGP / PEt	PEt	BGP / PEt	PEt	BGP / PEt	PEt	BGP / PEt
Liver	9	69	17	7.4	9	16	52	5.8
Kidney	1	1246	1800	3.3	41	6	480	4.5
Heart	6	24	160	3.5	1	78	19	2.0
Spleen	17	56	40	3.3	8	69	300	2.7
Brain	12	14	73	3.2	9	14	24	4.9
Muscle	12	15	18	1.3	23	7	23	1.0
Reticulum sarc.	3	75	57	4.3				
Walker 256 Ca.	1	364	32	4.6				
Human prostate	9760	3	38	4.0				

BGP = Sodium beta-glycerophosphate. PEt = Phosphoethanolamine.

ethanolamine nevertheless this tissue contained the most active phosphatase for splitting of phosphoethanolamine of any tissues examined, irrespective of pH.

Rat liver homogenate gave rather low Q values with phosphoethanolamine; however these were approximately 7-fold when beta-glycerophosphate was the substrate. Further investigation showed that dialysis of liver homogenate increased hydrolysis of phosphoethanolamine and glycerophosphate 100 and 50% respectively. Furthermore, dilution of liver homogenate of 1:10 increased the Q value 4-fold; whereas similar dilution of kidney homogenate caused no change in the hydrolysis rate. Finally, it was found that boiled, undialysed liver extract inhibited hydrolysis of phosphoethanolamine by rat kidney homogenate at pH 9.0. Dialysis of liver extract removed this inhibition, but concentration of the dialysate followed by addition to kidney homogenate restored the inhibition.

Discussion. Comparison of Tables I and II indicates there is little correlation between amounts of free phosphoethanolamine in a given tissue as compared to amounts of enzyme which would split the compound, *i.e.*, rat kidney and brain had approximately the same amounts of free ester, *viz.*, (.48 *vs.* .50); however kidney was about 25 times as active as brain at pH 9.0 in hydrolysing phosphate from the compound.

Our findings shown in Table II are in agreement with those of Roche and Bouchilloux (7) who had shown that phosphoethanolamine is split at an acid pH although at a lower rate than glycerophosphate. Our results are at variance with Strickland *et al.*(8) in that we found rat and rabbit brain homogenate to be fully as active under acid conditions toward phosphoethanolamine as most other tissues of these species. We also differ in that we found human prostate to contain the most active phosphatase of any tissue examined; whereas Strickland *et al.*(8) reported almost complete inactivity of this tissue toward phosphoethanolamine under acid conditions.

The increased Q value obtained with dilution and dialysis of liver homogenate suggests that the activity of this tissue phosphatase toward phosphoethanolamine might be obscured by a transphosphorylation reaction. Suitable aliquots of the remaining hydrolysate were chromatogramed on paper and showed equal quantities of ethanolamine and inorganic phosphate released with corresponding disappearance of a similar amount of phosphoethanolamine. Schmidt *et al.*(12) have shown that intestinal phosphatase is inhibited by phosphate ion. However, addition of .0075 mM phosphate, an amount equivalent to that found in 1 ml of liver homogenate, had no effect on activity of liver phosphatase on phosphoethanolamine. These results indicate that rather than a transphosphorylation, or inhibition by phosphate ion, there is perhaps a competition for the phosphatase between phosphoethanolamine and other phosphate esters present in liver.

Summary. 1. Optimum conditions for hydrolysis of phosphoethanolamine by tissue homogenates have been determined to be: veronal-acetate, pH 4.5, and veronal, pH 9.0, Mg^{++}, 5×10^{-3} M. 2. Concentration of phosphoethanolamine was determined in 6 rat and rabbit tissues as well as 2 transplantable tumors. 3. Alkaline phosphatase of the various extracts readily hydrolysed the ester and at greater rates than acid phosphatase. 4. Phosphoethanolamine was more readily hydrolysed by acid phosphatase of human prostate than of any other tissue examined. 5. Dilution and dialysis experiments with liver indicate there is a competition for phosphatase between phosphoethanolamine and other phosphate esters present in liver.

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Effect of Properdin on Whole Body Irradiated Mice and Rats.* (24492)

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The parenteral administration of properdin of heterologous origin into rats and mice has been reported to protect significantly against $LD_{100}/30$ day and $LD_{100}/6$ day doses of whole body x-irradiation(1,2). Degree of protection was reported to be dependent on dose, route, and time of administration of properdin. Ross(2) observed that mice exposed to 600 r and treated with 50 units of partially purified bovine properdin administered intravenously showed a 4-fold greater 30 day survival rate than the untreated controls. However, mice treated with properdin on the second, third, fourth, seventh, or tenth postirradiation days showed similar or accelerated death rates as compared to the controls. In order to further assess the role of properdin in protection of animals against the effects of whole body x-irradiation, experiments were conducted to determine the value of properdin as a therapeutic agent.

Materials and methods. Adult albino mice and adult albino rats (Sprague-Dawley) were randomly arranged into groups of varying numbers according to the design of each experiment. Dosage of x-rays administered for each experiment is given in the section on results. The x-irradiation was administered from above the animals by means of a Westinghouse Quadrocondex x-ray machine. The technical factors were: 250 KVP; 15 ma, 1 mm aluminum and 0.5 mm copper filters in addition to inherent filtration of 2.5 mm aluminimum and 0.25 mm copper; size of field, whole-body; target distance, 16 inches. The mice were irradiated in groups of 10 in a cylindrical container having a diameter of 18.5 cm and a height of 3 cm. The rats were irradiated in groups of 4 in a cylindrical container having a diameter of 23 cm and a height of 5.5 cm. Average dose rate as measured with a Victoreen r meter was approximately 90r per minute when recorded in the center of a lifelike wax phantom. Variation in radiation dose did not exceed 2r per minute when measured in any position in the container. Properdin solutions were prepared from fresh bovine serum according to the zymosan adsorption technic of Pillemer *et al.*(3) and also by a modified Cohn ethanol fractionation procedure. In addition, purified human properdin was used in the experiments.† The titers of the partially purified properdin (PPP) solutions and the human properdin (HP) were determined by the zymosan assay technic described by Pillemer *et al.*(3).‡ Route and time of administration of the properdin solutions is given in each experimental design.

Results. In the first experiment, 5 randomly selected groups of mice were exposed to a single 550 r total body dose of x-irradiation. Animals of the first group of 11 mice were in-

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