been possible with these substances to protect either mice or guinea pigs from anaphylaxis. In fact, BAS actually seems to enhance anaphylactic deaths.† For these reasons, it is premature to assume that pertussis cells exert their anaphylactic-enhancing effect by increasing the sensitivity to serotonin. It should be noted, however, that the type of death observed in pertussis-treated mice after injection of lethal doses of serotonin or histamine is grossly indistinguishable from that observed in anaphylaxis(13). Typically, after injection of serotonin mice become lethargic. Respiration becomes forced and within a few minutes signs of cyanosis are observed as evidenced by the color of snout, tail, ears and paws. The snout actually appears to be swollen. Little cyanosis is observed in non-lethal reactions. Mice usually die within 15-30 minutes giving few tonic contractions of the hind legs. Usually after these final convulsions mice continue to gasp for a short time. The similarity of death produced by these three different agents may well point to a common mechanism, and as Herxheimer(8) has suggested, death may result from a still unknown mechanism triggered by antigen-antibody reactions as well as by serotonin, histamine and perhaps other substances.

Summary. (1) Two strains of mice known to differ with respect to their ability to become sensitized to histamine, were found to become highly sensitive to serotonin after the injection of *H. pertussis* cells. (2) Some sensitivity to serotonin could be detected 16-24 hours after the administration of pertussis. Sensitivity reached its peak by the third to fourth day, and disappeared by the 25th day after pertussis injection. (3) Ability to induce sensitivity to serotonin seems to be characteristic of *H. pertussis*.

† Unpublished observations.

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Cytotoxic Effect of Nephrotoxic Serum on Rat Tissue Culture.* (23210)

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Lippman(1) reported that gamma globulin made from rabbit anti-rat-kidney serum inhibited growth in tissue culture explants of rat heart muscle and kidney as well as chick-embryo-brain, heart muscle and mesonephrons. The cytotoxicity of this serum did not appear species specific. Investigation of this problem has been renewed in our laboratory using monolayer tissue culture cells(2). This report concerns the effects of anti-rat-

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kidney serum on (1) rat kidney cells *in vivo* (nephrotoxicity), (2) rat kidney cells in tissue culture, (3) hemagglutination test and (4) the interrelationships.

Methods. Preparation of tissue culture. Kidneys of rats or other species were minced and trypsinized according to Youngner's technic(2). The renal cells were suspended in a medium containing 10% inactivated calf serum and 0.5% lactalbumin in Hanks balanced salt solution. One ml aliquots of this cell suspension were delivered into tissue culture tubes. After 3-4 days of incubation, the tubes were refed. On the same or the next day of refeeding, varying concentrations of antisera were added to the medium to give final dilutions of the serum from 1:6 to 1:192 in 2-fold steps. *Production of immune sera.* New Zealand rabbits weighing 2-3 kg each were used in pairs for producing various antisera against: 1. Saline organ emulsions: Perfused rat kidney, liver, lung and heart were macerated and prepared as 20% suspensions in physiological saline. They were given intraperitoneally by a schedule similar to Ehrich, Forman and Seifter(3). 2. Tryptic digest of rat kidney: This material was prepared by the method described by Cole(4). The material thus prepared had a total nitrogen content of 263 mg%. A total amount equivalent to 7-9 g of the original organ was given to each animal intravenously over a period of 8-10 weeks. A more concentrated tryptic digest of kidney was made with 500 mg% of nitrogen. This was injected intraperitoneally once a week over a period of 7 weeks with a total dosage equivalent to 21 g of original kidney tissue. 3. Glynn's antigen of kidney digest adsorbed onto streptococci: Streptococci of group A type 12 and concentrated rat tryptic kidney digest was used in the preparation as described by Glynn and Holborow(5). 4. Human kidneys: Normal infant kidneys obtained shortly after death were emulsified in a Waring Blendor. The 20% kidney emulsion was injected into rabbits intraperitoneally. Human kidney obtained at surgery was immediately planted into tissue culture bottles(2). After several passages, the sheets of cells were washed and collected. Five ml of a 5% cell suspension in saline was injected 3 times intraperitoneally and 6 times intravenously into rabbits in 6 weeks. All the above animals were bled from the heart 4-10 days after the last injection. The antisera were inactivated at 56°C for 30 min., adsorbed once with washed rat blood cells and then stored at –20°C until used. *Definition of cytotoxicity.* After antisera were added, renal tissue cultures were observed for cellular damage by low power microscopy daily for a period of 5 days. Cytologic changes were as follows: Mild or early effects were apparent as clumping of cells, nuclei became pyknotic and the cytoplasm became "dusty" and coarsely granular. There was shrinking of the cytoplasm of these cells sometimes leaving thready spicules extending from one cell to another. Dead and pyknotic cells appeared first along the margins of the sheets. This process continued until all cells disappeared from the walls of the culture tubes. We arbitrarily defined unequivocal cytotoxicity as the loss of at least 50% of the cells (as compared to control culture tubes) and alterations of the type detailed above in the remaining cells. *Method of absorption.* To each 2 ml of nephrotoxic sera, 0.75 ml of organ material was added separately. The materials used were digests from 0.4 g wet weight of rat lung, liver, heart and kidney and 50% whole kidney emulsion. The nephrotoxic serum was adsorbed with living rat kidney tissue culture cells by replacing the culture medium with 1 ml of nephrotoxic serum and then incubating at 37°C overnight. This was repeated 6 times. *Hemagglutination test.* Boyden's method(6) was adopted with slight modification in that 1:10,000 tannic acid and 5% sheep red cells were used. The tryptic digest antigen used for adsorption onto the tanned sheep blood cells contained approximately 2.5 mg N/ml. *Bioassay of nephrotoxic sera.* Long-Evans rats, weighing 100-150 g each, were used. For the rabbit anti-rat-kidney sera, 0.1-1 ml of serum was given intravenously per rat in a single or 2 divided doses. The other organ antisera were also given intravenously as 0.5-3 ml per rat in a single or 2-3 divided doses on 2-3 consecutive days. Eighteen hour urine specimens were collected on 1, 3, 7, 10 and 14 days

TABLE I. Comparison of Anti-Kidney Antibody Titers, Nephrotoxicity and Cytotoxicity of Various Antisera.

Rat tissue used for immunizing rabbit	Hemagglutination titers *vs*				Nephrotoxicity in rats	Cytotoxicity for rat renal tissue culture
	Kidney	Liver	Lung	Heart		
Kidney emulsion	40,960	<40	<40	640	++++	96*
" digest, I.V.	640	"	"	160	0	6
I.P.	160	"	"	<40	0	<6
Kidney digest adsorbed onto strep.	2,560	"	"	"	+++	24
Lung emulsion	640	"	40,960	640	0	6
Liver "	<40	40,960	2,560	"	0	<6
Heart "	"	160	<40	"	0	"
Streptococci	"	<40	"	<40	0	"
Normal rabbit sera and zero time	"	"	"	"	0	"

* Reciprocal of highest significant serum dilution.

after the last injection. The rats which survived until the end of the 2 weeks of observation were sacrificed. Histological examination of all kidneys was made by Dr. Bernard Wagner, associate pathologist of the hospital.

Results. From Table I, it is evident that the tryptic digest of rat kidney was only weakly antigenic. By adsorbing the soluble kidney digest onto streptococci, the kidney digest was apparently converted into a better antigen. The most potent nephrotoxic sera *in vivo* were those produced by injecting whole kidney emulsion. This was true in all parameters. Their anti-kidney hemagglutinin titers were highest, and they showed cytotoxic effects at 1:96 dilution of the sera in rat renal tissue culture.

The organ specificity of this cytotoxic effect is indicated by the results of similar tests with the other rat organ antisera. Only the anti-lung serum demonstrated slight cytotoxic effects. Normal rabbit sera (including inactivated sera of those rabbits used for producing various antisera obtained prior to period of immunization) were not cytotoxic.

Organ specificity was assayed further by comparing the adsorbing ability of standard doses of tryptic digests of various organs (Table II). While the amount of kidney digest used in this adsorption was insufficient to completely neutralize the nephrotoxicity and cytotoxicity of the nephrotoxic serum it did neutralize the antiserum made by injecting rat kidney digest adsorbed on streptococci. Lung digest was much more effective than digests of liver and heart in lessening reactivities in all parameters. The nephrotoxic serum, after adsorption with living rat kidney culture cells, was no longer cytotoxic in tissue cultures or nephrotoxic when injected in dose of 0.5-1 ml into rats, and the anti-kidney hemagglutinin titer was greatly reduced, (1:160).

Species specificity was demonstrated by using: (1) the usual nephrotoxic serum (for rats), (2) rabbit antiserum prepared against human kidney cells grown in culture and (3) rabbit antiserum prepared against whole human kidney emulsion. These sera in various concentrations were tested for cytotoxic effects in cultures of kidney cells derived from 6 species of animals. The nephrotoxic serum appeared most toxic for Long-Evans rat kidney cells and slightly toxic for white rat and DBA mouse kidney cells (Table III). However, it was nontoxic for other kidney cells. The antiserum against human kidney tissue culture cells was cytotoxic for human cells and weakly cytotoxic for monkey kidney cells. The antiserum against human kidney obtained from autopsy had toxic effects only for human kidney tissue culture cells.

Discussion. A correlation has been demonstrated to exist between anti-kidney hemagglutinin titers and *in vivo* nephrotoxicity in

TABLE II. Assay of Organ Specificity by Absorption Test.

Serum and added component	Anti-kidney titer (HA)	Nephrotoxicity in rats	Cytotoxicity in tissue culture
1. Anti-rat-kidney serum			
+ physiological saline	1:40,960	++++	1:96*
+ rat kidney emulsion	<1: 40	0	<1: 6
+ " " digest	"	+	1: 6
+ " lung "	1: 640	++	1:24
+ " liver "	1: 1,280	++++	1:48
+ " heart "	1: 2,560	++++	1:48
+ " kidney tissue culture cells	1: 160	0	<1: 6
2. Anti-strep.-adsorbed-kidney-digest serum			
+ physiological saline	1:2,560	+++	1:24
+ rat kidney emulsion	<1: 40	0	<1: 6
+ " " digest	"	0	"

* Dilution of sera demonstrating unequivocal cytotoxicity for rat kidney tissue culture cells.

these studies. Those sera demonstrating maximal nephrotoxicity had the highest titers of hemagglutinins. Sera lacking nephrotoxic effects in general had hemagglutinin titers of kidney antigens of less than 1:640. These results are in agreement with the recent report of Rothenberg, *et al.*(7). The range of hemagglutination titers also correlated well with the *in vitro* cytotoxic effects. Absorption of the nephrotoxic sera by digests of lung, heart and liver revealed a disproportional reduction in hemagglutinin titers in comparison to the neutralizing effect on nephrotoxicity and cytotoxicity. This would seem to indicate that the antigen-antibody systems measured by the hemagglutination system may differ in some respects from those responsible for nephrotoxicity and cytotoxicity.

We have also employed Middlebrook's(8) method of adsorbing the soluble tryptic digests of organs on sheep red cells not treated with tannic acid. Results with such cells in hemagglutination test were consistently negative. This suggests that the antigen-antibody system being measured by this test does not involve polysaccharide antigens of the kidney.

Baxter and Goodman(9) found that the nephrotoxic antibodies could be removed by adsorption of nephrotoxic sera with extracts of rat organs other than kidney though the quantity needed varied with the different organs. The results of the comparison of the neutralizing potency of the various organ preparations we used for adsorption of nephrotoxic sera are in agreement with their finding. Though these results can be interpreted as indicating some degree of organ specificity, they also could reflect differences in the quantitative distribution of a common antigen in the various tissues.

The data presented suggested that the one or more antibodies causing nephrotoxicity in the intact animals may be identical with the one or more antibodies causing cytotoxicity in culture. The evidence for this is based on the correlation between the cytotoxicity and the nephrotoxicity of the sera and the elimination of these effects by absorption of the sera with

TABLE III. Species Specificity of Cytotoxic Effect.

	Source of kidney tissue culture cells					
Rabbit antiserum against:	Long-Evans rat	White rat	DBA mouse	Rabbit	Monkey	Human
Rat kidney emulsion	1:96	1:24	1:12	<1:6	<1:6	<1: 6
Human kidney tissue culture	<1: 6	<1: 6	<1: 6	"	1:6	1:48
Human (autopsy) kidney emulsion	"	"	"	"	<1:6	1:24
Rabbit serum control*	"	"	"	"	"	<1: 6

* Sera obtained from rabbits prior to production of various antisera.

either whole kidney emulsion or kidney tissue culture cells. The cytotoxic effect in tissue culture of nephrotoxic sera appears to be species specific, though there is some cross reaction between closely related species of animals. This is significant in view of the species specificity of nephrotoxic serum nephritis (9).

Latta and Kutsakis(10) recently reported that a heat-labile, complement-like factor present in guinea pig anti-chick-embryo-heart serum was necessary for the immediate cytotoxic effect on chick-embryo-heart fibroblast grown in tissue culture. We are currently studying the effect of complement-like factors in this cytotoxic system.

Summary. Rabbit antisera to both rat kidney saline homogenate and the tryptic digest of rat kidney adsorbed on streptococci caused cytotoxic effects upon rat kidney cells in tissue culture as well as renal pathology *in vivo* following intravenous injection of rats. The titers of the cytotoxic effect in tissue culture paralleled the nephrotoxic effect *in vivo* and the anti-kidney hemagglutinin titers *in vitro*. The kidney cells grown in tissue culture tubes were effective in absorbing the potent nephrotoxic factor from serum. The cytotoxic effect of the nephrotoxic serum appears to be markedly species specific.

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A Plate Technic for the Conglutinative Complement Fixation Test. (23211)

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The term conglutination (*L. conglutinatio*, act of glueing together) was applied by Bordet and Streng(1) to describe clumping of red blood cells sensitized with antibody in the presence of complement (C′) and of a substance called conglutinin, existing in serum of normal bovines. Realizing that C′ was necessary for conglutination, Streng(2) developed a new serological test applied to the diagnosis of numerous diseases such as syphilis, glanders, dourine, brucellosis, etc. Interest in this test was revived recently largely as a result of the investigations of Hole and Coombs(3-6) and of Rice *et al.*(7,8). Hole and Coombs(3) suggested the expression "Conglutinating Complement Absorption" for Streng's serodiagnostic test to indicate the analogy to and difference from the hemolytic complement fixation (C′F) test. However, use of the word absorption instead of fixation does not seem entirely justifiable, since the same basic phenomenon, *i.e.* disappearance of C′, is involved in both cases. We shall refer,

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