were always performed first. After 48 hours' incubation at 37°C all fluids were tested for hemagglutinins. Table I shows the results of several experiments in which the 50% end-points of infectivity obtained with the two methods were calculated by the method of Reed and Muench.(4) It is seen that the titers of allantoic fluid as measured by allantoic sac inoculation were always lower than those obtained with the prepared membrane alone. Since a much larger amount of fluid is introduced into the "empty" eggs, a correspondingly higher amount of virus is also inoculated. This may explain the greater sensitivity observed. Other explanations seem possible.

Discussion. The method may have various applications. It is possible to introduce larger amounts of fluid into the egg than by other methods and this may be of advantage when virus isolation from throat washings are undertaken. The influence of fluids of different composition on virus multiplication and the effect of inhibitors and accelerators of growth can easily be studied by this method which allows virus growth in a practically intact tissue. Moreover, the rate and character of growth of virus in the membrane may be more accurately studied. Such experiments are in progress. The method may have certain advantages for the production of vaccines as virus multiplication can be obtained in a medium of chosen composition, and the presence of urates in the virus fluid is excluded.

Summary. A method is described in which the chorioallantoic membrane of the egg *in situ* is used for the cultivation of influenza virus. The sensitivity of the method is demonstrated and various possibilities for its application are discussed.

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Effect of Proteinuria on Toxicity of Mercurial Diuretics in the Rat. (17542)

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During the past 30 years, organic mercurial compounds have been used widely for the purpose of inducing diuresis. Because of their apparently low clinical toxicity, mercurial diuretics have been used with impunity in the presence of cardiac failure, and, even in the presence of impaired renal function, few clinicians have hesitated to administer them. Studies in animals(1-4) gave results which, while in some degree conflicting, nevertheless indicated that the agents could produce transient or permanent renal damage in doses which were comparable to those recommended for use in man. When clinical application of the mercurial diuretics was begun, it soon became evident that, although there was some indication of renal irritation,(4-6) the clinical toxicity was negligible,(7,8) at least in the absence of severe,

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pre-existing renal damage. It was determined soon after their introduction that mercurial diuretics acted directly upon the kidney, (9-12) and there has been general agreement that diuresis results from depression of tubular function. The localization of this action in the tubule has been studied more recently, but has not been completely clarified. There are some indications that the effect is produced upon the proximal tubule,(13,14) whereas some evidence indicates an effect upon the distal tubule.(15) To further complicate matters, there is evidence for species differences in the effects of mercurial diuretics.(13,16)

Because it seemed probable that mercurial diuretics are potentially toxic substances which act by depressing tubular function, it seemed remarkable that such toxicity does not become clinically evident in patients with the nephrotic syndrome, when mercurial diuretics are used. Here, in a situation which is accompanied by tubular degeneration, it would seem reasonable to suppose that damaged tubule cells, seen as desquamated cells in the urinary sediment, would be more susceptible than normal cells to the toxic action of these compounds. Yet such patients often receive mercurial diuretics without ill effect, though often, in our clinical experience, without therapeutic effect. As a result of such observations and reflection, we became interested in studying the effect of proteinuria upon the toxic action of mercurial compounds.

TABLE I.
Effect of Proteinuria on Kidney Weight and Body Weight After Administration of Mercurial Diuretic.

Group No.	IP inject.	No. rats	Mercurial used	Mercurial inj. after IP inj. No.	Mercurial dose, mg Hg	No. rats surviving	Time kill interval survivors, days	Initial wt, g	Final wt, g	Kidney wt, mg	Anatomic diagnosis
1	Saline	6	Mersalyl	III	.26	6	1	153		1137	Nephrosis, acute, very slight
1a	Albumin	6	"	III	.26	6	1	154		1226	Nephrosis, acute, very slight
2	Saline	12	"	III	.79	12	1	158		1103	Nephrosis, necrotizing, acute, severe
2a	Albumin	12	"	III	.79	12	1	159		1273	Nephrosis, acute, slight to moderate
3	Saline	14	Mercallur.	III	1.95	10	7	153	134	1635	Nephrosis, acute, severe, advanced healing
3a	Albumin	13	"	III	1.95	13	7	152	155	1183	Nephrosis, acute, slight
3b	"	6	"	II	1.95	6	7	154	154	1167	Nephrosis, acute, very slight
3c	"	6	"	I	1.95	1	7	150		2561*	Nephrosis, acute, severe, healing, slight hydronephrosis

* One surviving animal only.

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Methods and materials. This study was performed with 75 female rats, each weighing about 150 g. The organic mercurial diuretics studied were mersalyl-theophylline and meralluride sodium. Mersalyl-theophylline contains 39.6 mg Hg and 50 mg theophylline in 1 ml. Meralluride sodium contains 39.0 mg Hg and 48 mg theophylline in 1 ml. In the dosages tabulated (Table I) theophylline was present in the above ratio to the dose of Hg. The animals each received three 16 ml intraperitoneal injections, No. I at 9:30 A.M. and No. II at 4:30 P.M. on the first day, and No. III at 9:30 A.M. on the second day. For the control groups, the injection material was 0.85% sodium chloride solution. For the experimental animals, the injection material was 6% human albumin in 0.85% sodium chloride solution. Immediately after the designated intraperitoneal injection (Table I), the mercurial diuretic was administered intravenously, diluted to give the proper dose with 0.85% sodium chloride solution. The animals were then left undisturbed upon stock diet either until death, or for the interval specified, at which time they were killed by ether anesthetization and exsanguination from the abdominal aorta. The kidneys were removed promptly, except in the few animals that died during the night, decapsulated, sagittally sectioned, blotted, weighed, and dropped into 10% formalin. After fixing, paraffin sections were made and stained with hematoxylin and eosin for microscopic examination.

Results. The animals in Group 1 (saline + 0.26 mg Hg) and 1a (albumin + 0.26 mg Hg) appeared perfectly normal until killed. At autopsy they also appeared perfectly normal. There was no appreciable difference between the groups in kidney weight, except for the difference expected after albumin administration. The kidneys appeared normal in the gross, both externally and on sagittal section. In the microscopic sections there were minimal changes of acute nephrosis present in both groups. There was no convincing difference in the degree of nephrosis between the two groups. The animals in Group 2 (saline + 0.79 mg Hg) and 2a (albumin + 0.79 mg Hg) also appeared normal until killed. At autopsy, as in all the animals, there were no significant findings outside of the kidneys. In Group 2 the kidneys were somewhat smaller than normal, with slight pallor of the surface and the cortex. In Group 2a the kidneys were normal in size and the pallor was hardly evident, resembling the slight pallor that accompanies protein injections alone, in our previous experience. Microscopically, the kidneys in Group 2 showed definite and pronounced tubular damage (Fig. 1), while those in Group 2a showed slight damage of a much milder degree (Fig. 2).

In the animals of Group 3 (saline + 1.95 mg Hg), the findings were entirely different. These animals were obviously ill from the day of the mercurial injection and 4 of the 14

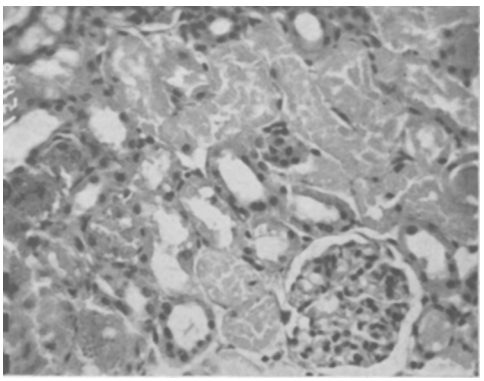

FIG. 1.
Section of kidney, Group 2: saline-injected animals, 1 day after mersalyl (0.79 mg Hg), showing acute necrotizing nephrosis. Hematoxylin-eosin stain, 240×.

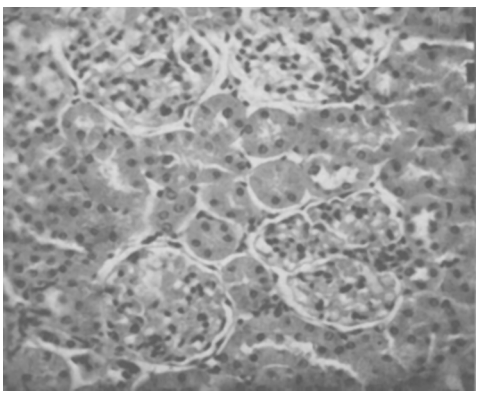

FIG. 2.
Section of kidney, Group 2a: albumin-injected animals, 1 day after mersalyl (0.79 mg Hg), relatively free of tubular damage. Hematoxylin-eosin stain, 240×.

animals died from the fourth to the sixth day after injection. At the end of a week, the survivors were severely ill, emaciated, and hyperirritable when disturbed. In this group the kidneys were greatly enlarged, with extremely pale granular surfaces. On sagittal section, the cortex was very pale and striated, while the medulla appeared to be relatively well preserved and of almost normal color. On microscopic examination, the tubular structure was disrupted, with appearance of severe toxic nephrosis in an advanced healing stage. (Fig. 3, 4). In sharp contrast, the animals in Group 3a (albumin + 1.95 mg Hg) appeared to be entirely normal until

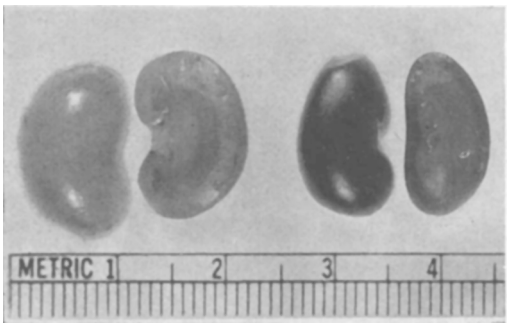

FIG. 3.
Kidneys, gross specimens. Left: Group 3, saline-injected animals, 7 days after meralluride sodium (1.95 mg Hg). Right: Group 3a, albumin-injected animals, same interval and same dose of meralluride sodium, injected after albumin injection No. III. Note difference in size and color of kidneys.

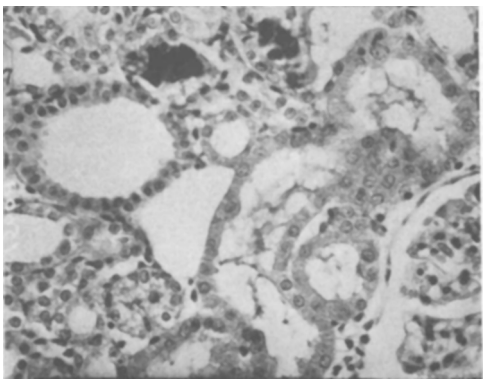

FIG. 4.
Section of kidney, Group 3: saline-injected animals, 7 days after meralluride sodium (1.95 mg Hg), showing necrotizing nephrosis with healing and deposits of calcific material. Hematoxylin-eosin stain, 240×.

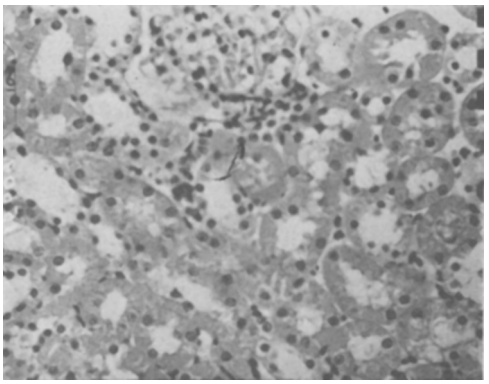

FIG. 5.
Section of kidney, Group 3a: albumin-injected animals, 7 days after meralluride sodium (1.95 mg Hg), administered at time of albumin injection No. III. Note relatively intact tubular structure with mild changes of acute nephrosis. Hematoxylin-eosin stain, 240×.

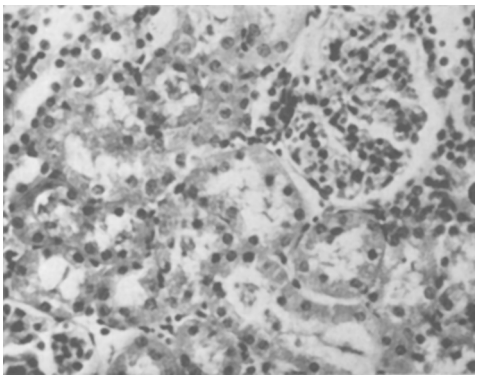

FIG. 6.
Section of kidney, Group 3b: albumin-injected animals, 7 days after meralluride sodium (1.95 mg Hg), administered at time of albumin injection No. II. Note that degree of damage is relatively slight, similar to that in Fig. 5. Hematoxylin-eosin stain, 240×.

killed. All survived in good health and there was a slight gain in weight. At autopsy, the kidneys were normal in size and appearance except for the slight pallor mentioned under Group 2a. Microscopically there was a very slight degree of tubular damage (Fig. 5). In Group 3b (albumin + 1.95 mg Hg after inject. No. II) the animals were entirely comparable to those in Group 3a, and the kidneys were virtually indistinguishable (Fig. 6). The animals in Group 3c (albumin + 1.95 mg Hg after inject. No. I) were the most severely affected of all. Only one animal survived the

full week, and it appeared moribund. The kidneys of those dying earlier showed pronounced enlargement, but those of the one surviving were most remarkably enlarged, almost chalky white, with a granular surface. On sagittal section, the entire kidney was similarly without color in both medulla and cortex. Microscopically, the tubular damage was profound and more severe than in any other group (Fig. 7).

Discussion. The data given here show conclusively that intraperitoneal administration of albumin to rats, in large doses, *prior* to the administration of a severely toxic dose of mercurial diuretic, effectively protects the kidneys from the toxic effects of mercury. On the contrary, when the protein is administered simultaneously with the mercurial, the toxic effect of mercury is enhanced. Many previous experiments with administration of protein, in the same dosage used in this study, have established the relation of proteinuria to the time of injections.(17,18) The first appearance of proteinuria after such injections has been interpreted as indicating that the maximum tubular capacity for reabsorbing protein has been exceeded, so that the rate of protein reabsorption is critically diminished. This coincides with the time of our injection No. II. Therefore, it seems probable that the diminished toxicity of mercurial diuretic at this time is associated with diminished tubular reabsorption of mercury as well as protein. On the other hand, it seems plausible to suppose that, during the interval from the first protein injection to the first appearance of proteinuria, the reabsorption of protein proceeds at a more rapid rate than normal, and that during this period the reabsorption of mercury is similarly increased, so that the toxicity of a given intravenous dose is enhanced. That mercurial diuretic is selectively concentrated in the kidney, in patients who have received mercurial diuretics(19) and in normal dogs,(20) is already known. Little is known about the precise molecular structure and physical properties of the mercurial diuretics. The prosthetic, mercury-containing portion of the complex is unstable except in combination with theophylline. However, the precise nature of the combination is not known, nor is the size of the complex known. We have tried to dialyse meralluride sodium, and found that after 48 hours virtually none had passed through a cellophane membrane. In an effort to ascertain whether the mercurial diuretics are bound by serum protein, meralluride sodium was incubated with 6% human albumin and then separation was attempted by precipitating the protein, using trichloracetic acid, phosphotungstic acid, and phosphomolybdic acid. In each case, the meralluride sodium did not appear to be bound by protein, all remaining in the supernatant fluid. Therefore, it would appear that inhibition of mercury reabsorption is probably not related to a union between the mercurial diuretic and protein, as such. Experiments are in progress to determine by cytological demonstration whether our hypothesis, that proteinuria reduces the quantity of mercury reabsorbed, is correct.

From these findings, it is suggested that our clinical experience concerning the unex-

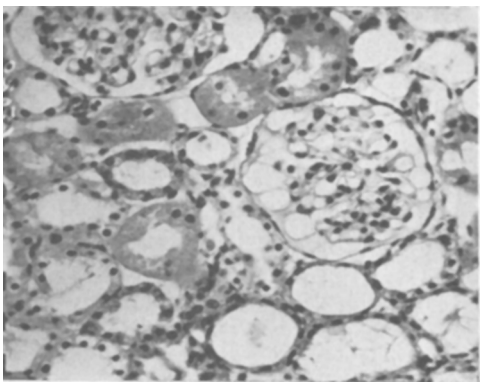

FIG. 7.

Section of kidney, Group 3c: albumin-injected animals, 7 days after meralluride sodium (1.95 mg Hg), administered at the time of albumin injection No. I. Note extreme severity of toxic nephrosis. Hematoxylin-eosin stain, 240×.

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pected relative innocuousness and therapeutic ineffectiveness of mercurial diuretics in patients with renal disease may be related to the presence of marked proteinuria.

Addendum. After submission of this paper, we became aware of work done by Havill, Lichty, and Whipple,(21) showing that the tolerance of dogs for mercury bichloride was increased by frequent hemoglobin injections. It seems reasonable to assume that the mechanism of protection from the toxic action of the metallic ion was similar in this instance, since hemoglobin, like albumin, is reabsorbed by the proximal tubule cells until saturation of the reabsorptive capacity occurs, although Havill and his co-workers did not relate the protective effect to the occurrence of hemoglobinuria, which they did not measure, suggesting instead that the pigmented moiety of the hemoglobin molecule was responsible for the protection.

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Summary. Administration of human albumin to rats, with resultant proteinuria, prior to administration of mercurial diuretics, sharply reduces renal toxicity of the mercurial. This effect is attributed to inhibition of mercurial reabsorption when the tubules are saturated with protein.

Simultaneous administration of human albumin and the mercurial enhances toxicity. This effect is attributed to the more rapid absorption of mercurial, concomitant with more rapid reabsorption of protein during the tubular loading phase.

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Fetal Death and Maldevelopment Resulting from Maternal Vitamin A Deficiency in the Rat.* (17543)

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The course of pregnancy may be severely altered by feeding female rats a diet restricted in vitamin A, the severity of the alteration being roughly proportional to the degree of vitamin deprivation. Death and resorption of the fetuses, prolongation of pregnancy, and delivery of stillborn young were reported by Mason(1) and Cannon(2) who employed deficient diets with little or no supplement of vitamin A or carotene. Warkany and Schraffenberger(3,4) using similar diets supplemented with small amounts of carotene also noted a high incidence of fetal resorption; but, by interrupting pregnancy at various times from the 13th day to term, they were able to obtain a number of living and recently dead fetuses and newborns suitable for histologic study. The eyes of these offspring were found to bear several developmental anomalies, notably: folding and eversion of the retina, persistence of the choroidal fissure, absence of the ciliary body, and postlenticular fibroplasia. Thus, deficiency of vitamin A in the diet of female rats prior to and during pregnancy not only reduces fecundity by

* Supported by a grant from the Nutrition Foundation, Inc., New York.

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