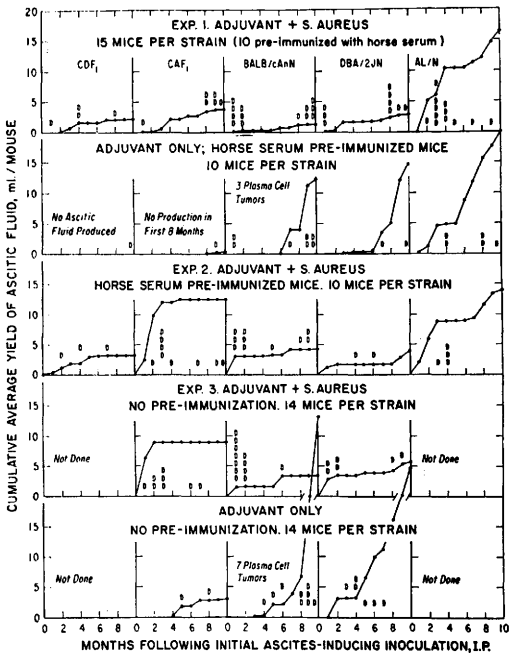

FIG. 1. Ascitic fluid production following inoculation with incomplete Freund's adjuvant alone or with heat-killed *S. aureus*. Cumulative average yields shown are based on total number of mice in experiment. Deaths of individual mice are indicated by letter *D*.

sooner and undue accumulation of fluid avoided. These alterations in technic might have led to higher incidence rates and perhaps also to higher yields than were observed.

Plasma cells have been implicated in antibody production(6) and if the property also resides in the plasma cell tumor, its occurrence in the BALB/cAnN mouse may provide a useful tool. A strain-specific carcinogenic role for the components of the adjuvant employed is suggested by the results for these mice.

Summary. Mouse strains vary in their production of ascitic fluid. Intraperitoneal injections of heat-killed *Staphylococcus aureus*-incomplete Freund's adjuvant mixtures induce early development of ascites. Yield of ascitic fluid may be greater with adjuvant alone but production is often delayed. Yield in individual mice may be very large. High late yield for BALB/cAnN mice receiving only adjuvant are associated with occurrence of plasma cell tumors.

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Red Cell Factor in Renal Damage from Hypertonic Solutions.* (26567)

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Previous work has demonstrated that the intravascular administration of certain solutions having a concentration greater than 1500 mOsm/L will produce red cell agglutination (1). This effect has been demonstrated in the lung, mesentery, brain and extremities of both cats and dogs(2). Removal of the red cell from the circulation to these areas abolishes the increase in vascular resistance which usually follows injection of markedly hypertonic media. Since the phenomenon of intravascular aggregation has been shown to be a cause of death in cardioangiography(3) and the mechanism responsible for ischemic arteriographic complications(4), we have been interested in determining its possible role in renal damage following aortography. As a first step, the following study was undertaken to delineate those changes in the kidney which result from osmotic activity alone.

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Methods. Adult mongrel dogs, 8 to 12 kg in weight, were anesthetised with 30 mg/kg of pentobarbital sodium. The aorta above and below the renal arteries was exposed through a midline abdominal incision. Vascular clamps were placed proximal and distal to this segment and upon the right renal artery, thus enabling that kidney to serve as a control. The left kidney was immediately perfused from a reservoir at a pressure of 150-200 mm Hg, through a needle inserted into the aortic segment. Flow rates were in the range of 30-60 ml/min. The perfusate was either autologous heparinized blood or plasma; the latter obtained 10 days previously by phlebotomy. One ml/kg of either 12.5% sodium chloride or 25% urea in 0.9% saline was injected rapidly 30 to 60 seconds after onset of perfusion. Total time to perfusion was 2 minutes or less in all cases. Vascular clamps were then removed and normal circulation restored to both kidneys. The aorta at point of injection was repaired with fine arterial silk, taking care that no constriction resulted. The animals were maintained for a period of 7 days under standard kennel conditions. Intravenous fluids were administered as necessary and each dog received antibiotics for 4 days following the procedure. On the seventh day they were sacrificed following intravenous pyelography, and the kidneys removed for gross and microscopic study.

A subsequent group was operated upon with one modification. The right ureter was ligated prior to perfusion so that the injected side would remain as the sole functioning renal tissue. Sodium chloride (12.5%) was injected as before during blood and plasma perfusion. Daily blood urea nitrogen determinations were made over the next 7 days followed by sacrifice.

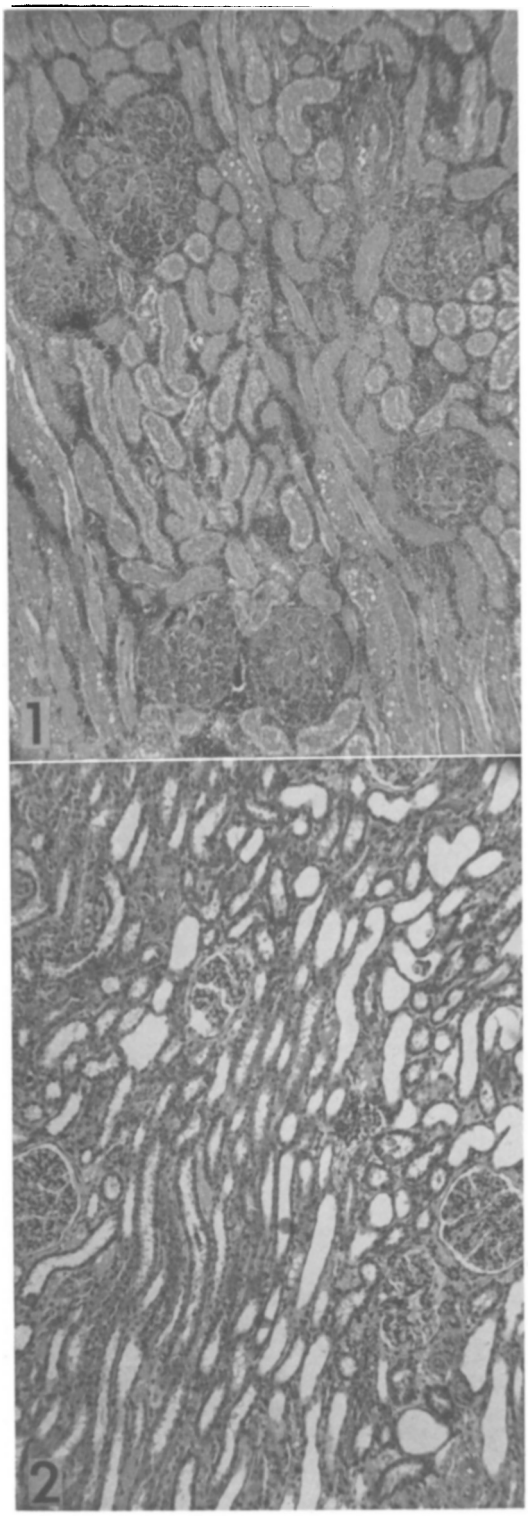
Results. A total of 36 animals were used in the experiment. Twelve received urea injections in the presence of either blood or plasma, and the remaining 24 were injected with 12.5% sodium chloride under the same conditions. Results in the injected kidneys have been divided into 2 groups depending upon which solution was used. In each instance the kidney which served as a control and was therefore not exposed to hyperosmotic materials concentrated well on intravenous pyelography, and was normal at postmortem examination.

Kidneys injected with 25% urea in 0.9% saline. Injections of 25% urea in 0.9% saline into both blood and plasma perfused kidneys failed to produce significant renal damage. All 12 animals were clinically well during the course of the experiment. Good visualization was obtained on intravenous pyelography and at autopsy. The only pathology noted was an occasional area of tubular vacuolization.

Kidneys injected with 12.5% sodium chloride. In those 12 animals whose kidneys were perfused with plasma, the results were comparable to those previously described for urea. However when blood was used, the dogs were listless, refused to eat and required parenteral fluid support. In no case was the injected kidney seen on intravenous pyelography. At autopsy, diffuse parenchymal hemorrhage with areas of infarction was apparent grossly, and severe renal damage had occurred microscopically (Fig. 1).

The function of kidneys injected with 12.5% sodium chloride was further studied in the modified group of 12 animals. All animals perfused with blood rapidly became uremic. Two died at 72 hr (see Fig. 3). The remaining 4 animals were toxic and exhibited no evidence of improvement to time of sacrifice. Microscopic and gross examination of these organs demonstrated damage similar to that previously described. However mean blood urea nitrogen values for the plasma perfused organs subjected to 12.5% sodium chloride did not rise above 40 mg %; no deaths occurred in this group and their postoperative course was benign.

Discussion. The results show clearly that hypertonic sodium chloride is more damaging to the kidney than an equally concentrated solution of urea. Further this damage can be significantly reduced by removing red cells from the renal circulation. These findings indicate that the renal toxicity of concentrated solutions is dependent to a considerable degree upon the presence of the red cell, rather than being primarily a direct effect upon the kidney substance. This conclusion is in di-



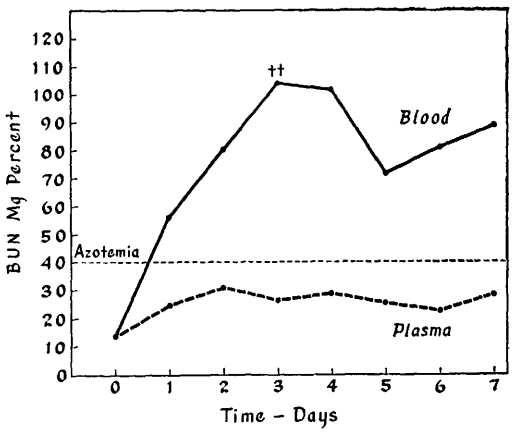


FIG. 3. Mean blood urea nitrogen values in animals injected with 12.5% sodium chloride during blood and plasma perfusion of their sole functioning kidney. Crosses mark the death of 2 animals at 72 hr.

rect opposition to recent work performed with concentrated organic iodide solutions; however the conclusions drawn from this latter investigation were based upon indirect evidence alone(5). It should be realized that the severe changes under consideration are superimposed upon certain microscopic alterations previously categorized as osmotic nephrosis(6). Tubular damage of this latter nature has been described after administration of a number of mildly hyperosmotic materials.

It is interesting to speculate upon the reason for the role of the red cell in the pathogenesis of renal damage from hyperosmotic solutions. The fact that concentrated urea as opposed to isosmolar sodium chloride did not cause necrosis is obviously important. This suggests, in view of the marked difference which exists in their ability to exert an osmotic differential across the red cell membrane, that osmotic damage to the red cell is the significant factor involved. Previous work in other areas of the body has demonstrated

FIG. 1. Renal changes present at 7 days in canine kidney subjected to 12.5% sodium chloride during blood perfusion. All renal elements are involved in the destructive process, which is in essence, an infarct.

FIG. 2. Renal changes accompanying administration of 12.5% sodium chloride during plasma perfusion 7 days following injection. Note retention of architecture, and tubular vacuolization typical of osmotic nephrosis. More severe changes consisting of tubular desquamation and cast formation are present in many areas.

that red cells subjected to a marked osmotic gradient agglutinate and thus produce obstruction to blood flow by a process of microthromboembolism. The present findings are consistent with the hypothesis that this phenomenon is responsible for renal destruction arising from concentrated solutions.

Summary. Laparotomy was performed on 36 mongrel dogs. The aorticorenal segment was isolated and perfused with either blood or plasma. One ml/kg of 12.5% sodium chloride or 25% urea in 0.9% saline was injected during the perfusion. Concentrated sodium chloride produced extensive damage in the blood perfused organ but not when plasma was used. Kidneys injected with 25% urea in 0.9% saline regardless of perfusate did not differ from control organs or from each other. Animals whose only functioning kidneys were injected with sodium chloride developed uremia if blood was used for perfusion. It is concluded that the renal toxicity of concentrated solutions is dependent to a considerable degree upon the presence of the red cell and is not therefore a primary effect upon the renal parenchyma. The importance of the red cell under these circumstances may be related to the phenomenon of intravascular agglutination which has been described previously in other areas of the body following administration of markedly hyperosmotic solutions.

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Mitochondrial Swelling Produced by Compounds Exhibiting Estrogenic Activity.* (26568)

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The observation that the synthetic estrogen, diethylstilbesterol, when given together with carcinostatic levels of the niacin antagonist, 6-aminonicotinamide, produces regression in the 755 tumor grown in C_{57} black mice(1) coupled with the demonstration that diethylstilbesterol greatly increases the ability of 6-aminonicotinamide to antagonize various pyridine nucleotide-dependent multienzyme systems(1,2) led us to determine the effect of diethylstilbesterol and other synthetic or natural phenolic compounds possessing estrogenic activity on mitochondrial volume or structure. The report of these findings is the purpose of this communication.

Materials and methods. Mitochondria were isolated from the livers of non-fasted male Holzman rats weighing 250-350 g. Preparation of the mitochondria and the technic employed in measuring mitochondria swelling were essentially that of Tapley(3) except that a Beckman DU spectrophotometer was employed. Inhibitory compounds of reagent grade were dissolved in 95% ethanol immediately before use and were shielded from sunlight. Ethylenediaminetetraacetic acid (EDTA), Mg^{++} and adenosine triphosphate(ATP) were dissolved in the concentrations indicated and adjusted to pH 7.4 before use. Three times glass-distilled water was used throughout.

Results. The stability of rat liver mitochondrial preparations in various concentrations of sucrose with and without diethylstilbesterol is presented in Table I. The results

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