Influence of Ionic and Nonionic Materials on Thermally-induced Ribonucleic Acid Degradation and Leakage in *Staphylococcus aureus*

M. C. ALLWOOD* and A. D. RUSSELL

Abstract □ Suspensions of *Staphylococcus aureus* were held at 50 or 60° in water, 1 *M* sucrose, or 0.5 *M* sodium chloride, and analyses were made of cellular ribonucleic acid (RNA), the content of RNA-like material in the acid-soluble (metabolic) pool and the amount of RNA-like material leaked. In sucrose and sodium chloride, as compared to water, RNA degradation was reduced at 50° and increased at 60°. In comparison to water, Mg^{++} ions reduced RNA-like material leaked at 37 and 50°, but not at 60°. The results are discussed in relation to the effect of thermal damage and loss of viability of the organism.

Keyphrases □ Ribonucleic acid (RNA) degradation, leakage—thermally induced □ Degradation, *Staphylococcus aureus* RNA—temperature effect □ Ionic, nonionic materials effect—RNA degradation □ UV spectrophotometry—analysis

Previous studies (1–4) have been concerned with the reasons for thermally-induced death in *Staphylococcus aureus* strain NCTC 3761. Several changes took place in heated suspensions of this organism: protein coagulation, a decrease in cell volume, a breakdown of ribonucleic acid (RNA), an increase in the content of RNA-like material in the acid-soluble (metabolic) pool and leakage of RNA-like material, damage to the cytoplasmic membrane, and leakage of amino acids. Deoxyribonucleic acid (DNA) did not leak from the cells and did not appear to be degraded. RNA degradation may be responsible for thermally-induced death in *Aerobacter aerogenes* (5), *Staphylococcus aureus* (3, 6, 7), and *Streptococcus faecalis* (8).

Some effects of sucrose on thermal death of *Staph. aureus* have also been described (3). These earlier findings are here considered in greater detail, and the effects of sodium chloride and Mg^{++} on RNA degradation or leakage are also presented.

MATERIALS AND METHODS

Materials—Magnesium sulfate, $MgSO_4 \cdot 7H_2O$, sucrose, and sodium chloride were of analytical reagent quality. Solutions were sterilized by heating in an autoclave.

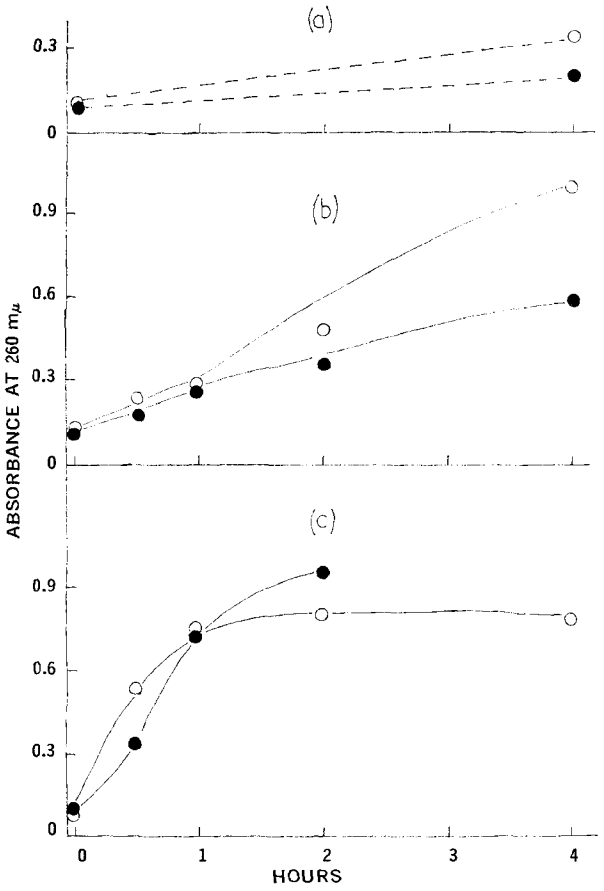

Figure 1—*Leakage of 260 mμ-absorbing material from suspensions of* Staph. aureus *stored in water* O—O *and 2* × 10^{-3} M *magnesium sulfate solution* ●—● *at* a *37°*, b *50°*, *and* c *60°*.

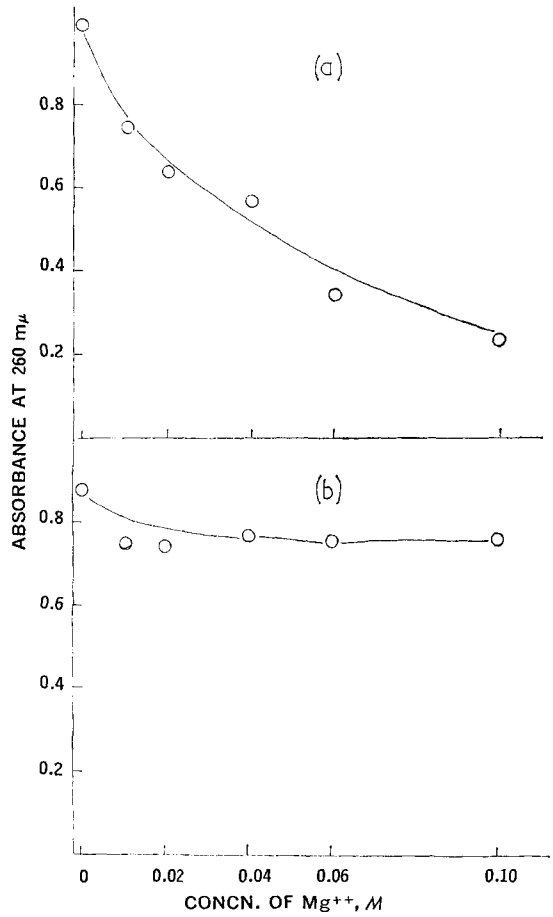

Figure 2—*Effect of magnesium sulfate concentration on the amount of 260 mμ-absorbing material from suspensions of* Staph. aureus *held for 4 hr. at* a *50°*, b *60°*.

Organism—*Staph. aureus* was grown for 18 hr. in nutrient broth (Oxoid Laboratories, Ltd., London, England), the culture centrifuged, the pellet washed twice with sterile water, and finally adjusted with sterile water to contain approximately 10^{10} viable cells/ml.

Heating Procedure—One part of a washed suspension was added to nine parts of the desired suspending medium, already at the required temperature (±0.1°), in a thermostatically-controlled water bath.

Viable Counts—Samples were removed at intervals, and viable counts made as described previously (3) using the pour-plate method, a plating medium consisting of nutrient agar containing 1% w/v yeast extract (Difco Laboratories, Ltd., Detroit, Mich.) and an incubation period of 48 hr. at 37°.

Analytical Procedure—The breakdown and release of RNA with *Staph. aureus* cells held at 50 or 60° in water, sucrose or sodium chloride were examined. An increase in the content of RNA-like material in the cold perchloric acid-soluble metabolic pool is indicative of breakdown of RNA. Details of the methods of obtaining the cold acid-soluble fraction, and of the RNA fraction, consisting of cellular RNA, *i.e.*, the RNA in the cell except for the small molecular weight RNA material in the metabolic pool, have been considered previously (3). Cellular RNA, RNA-like material in the pool, the RNA-like material leaked were determined by the orcinol technique (9) and by absorbance at 260 mμ, employing yeast RNA (British Drug Houses, Ltd., London, England) as standard. It has previously been found (1, 10) that closely related results were obtained in assaying RNA (or RNA-like material) by these two methods. Moreover, when sucrose was used as suspending medium, the orcinol technique could not be employed. When *Staph. aureus* cells were suspended in magnesium sulfate solutions, leakage of 260 mμ-absorbing material was measured.

RESULTS AND DISCUSSION

Mg^{++} ions are known to play an important part in membrane stability (11) and Strange (12) suggested that Mg^{++} stabilized bacterial membranes and reduced the lethal influences of chilling. It can be seen from Fig. 1 that, Mg^{++} reduced the amount of leakage at 37 or 50°. There appears to be no influence of Mg^{++} on leakage during storage at 60°, although the rate of loss of 260 mμ absorbing material may be reduced during the early stages of storage. It is apparent that Mg^{++} ions may influence the loss of intracellular material. The apparent lack of effect of Mg^{++} on leakage from cells stored at 60° was confirmed by storing cell suspensions in suspending fluids containing different concentrations of Mg^{++} for 4 hr. at 50 or 60°. The results are shown in Fig. 2. The total amount of 260 mμ-absorbing material lost from the cells is directly reduced at 50° as the concentration of Mg^{++} present is increased, but such a reduction is not observed at increasing Mg^{++} concentrations during storage at 60°. It seems unlikely that magnesium sulfate at the highest concentration employed could exert any osmotic influences to reduce the loss of intracellular material.

Mg^{++} ions are known to increase the stability of cellular ribosomes (13) and it was found that magnesium sulfate reduced the leakage of 260 mμ-absorbing material from heated *Aerobacter aerogenes* (5) and from starved *Sarcina lutea* (14), possibly due to stabilization of ribosomes. Thus, the apparent protective influences of Mg^{++} may be a result of the stabilization of RNA-containing units within the cell as well as the cell membrane. However, the variation in response of *Staph. aureus* stored in the presence of Mg^{++} at 50 and 60° is emphasized by the results presented here.

The changes in RNA in *Staph. aureus* cells suspended in water, 1 *M* sucrose, or 0.5 *M* sodium chloride are shown in Figs. 3–5. Cells in water at 50° (Fig. 3*a*) show a small but rapid initial decrease of cellular RNA, paralleled by a rise in the RNA-like content of the metabolic pool; this is followed by a slower loss of RNA-like

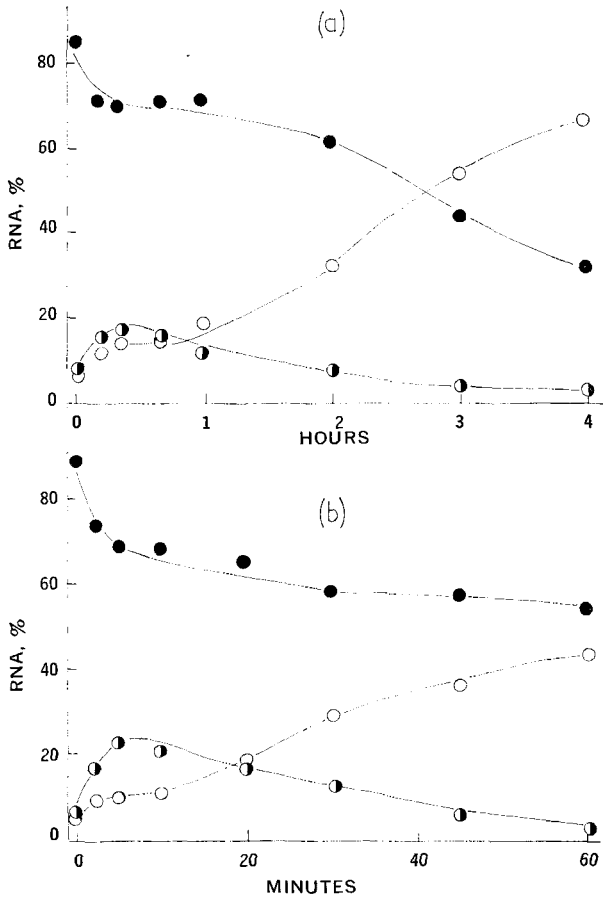

Figure 3—*Changes in the composition of RNA-like material in the metabolic pool* ◑—◑, *cellular RNA* ●—●, *and of RNA-like material leaked* ○—○, *with a suspension of* Staph. aureus *held in water at* a *50°*, b *60°*.

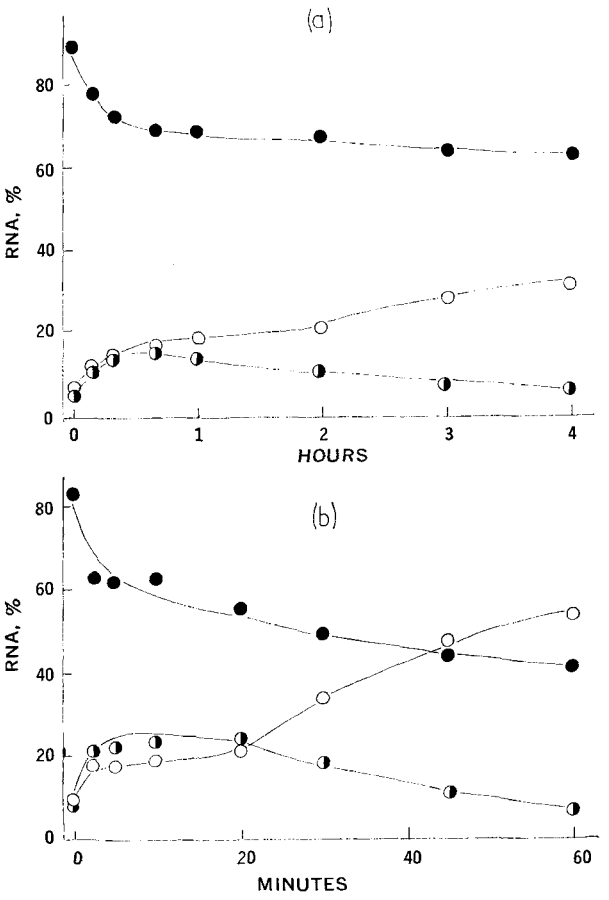

Figure 4—*Changes in the composition of RNA-like material in the metabolic pool* ◑—◑, *cellular RNA* ●—●, *and of RNA-like material leaked* ○—○, *with a suspension of* Staph. aureus *held in 1* M *sucrose at* a *50°*, b *60°*.

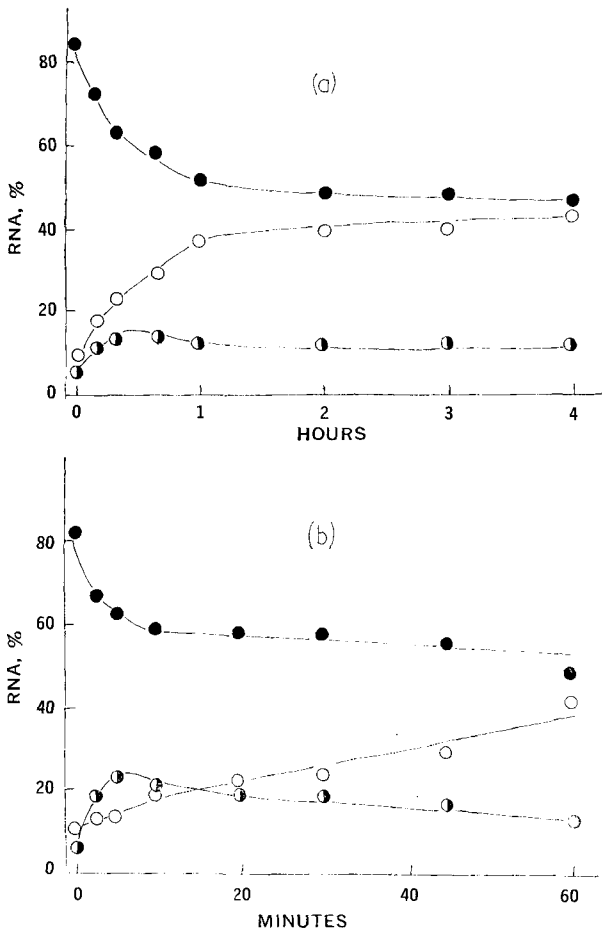

Figure 5—*Changes in the composition of RNA-like material in the metabolic pool* ◑—◑, *cellular RNA* ●—●, *and of RNA-like material leaked* ○—○, *with a suspension of* Staph. aureus *held in 0.5 M sodium chloride at* a *50°*, b *60°*.

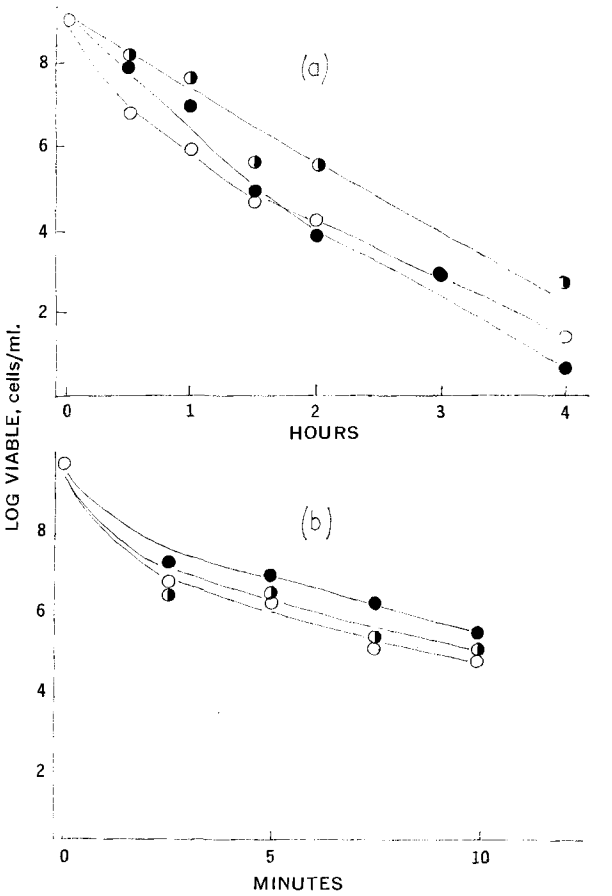

Figure 6—*Loss of viability of suspensions of* Staph. aureus *held in water* ○—○, *1 M sucrose* ●—●, *and 0.5 M sodium chloride* ◑—◑ *at* a *50°*, b *60°*.

material into the supernatant fluid, this loss clearly being derived from the degradation of cellular RNA and not principally from the cell pool. Subsequently, there is a decrease in the content of RNA-like material in the pool, and an increased leakage of RNA-like material into the surrounding environment; this diphasic loss of RNA-like material could be the result of enzyme action, as previously discussed (3), although there is no evidence as yet to substantiate this, or could be caused by leakage of large molecular weight RNA through the damaged cycloplasmic membrane. More delicate techniques are needed, however, since the reported techniques do not permit the authors to determine this.

Sodium chloride (Fig. 5*a*), like sucrose (Fig. 4*a*) protects *Staph. aureus* at 50° against the leakage of intracellular RNA-like material. It also appears that the loss of RNA-like material from the metabolic pool occurs more slowly in the presence of an osmotic stabilizer than when cells are held in water at 50°. The presence of 1 *M* sucrose as suspending medium appears to prevent the secondary breakdown (or leakage) of RNA; RNA degradation, or leakage, is higher when cells are held in saline, but again there is no diphasic pattern in the depletion of cellular RNA or in the leakage of this material.

At 60°, with *Staph. aureus* cells held in water (Fig. 3*b*), the pool content of RNA-like material increases rapidly, the content of cellular RNA falls, but leakage of RNA-like material occurs to a considerably slower extent. Subsequently, there is a decrease in the amount of RNA-like material in the pool, presumably as the membrane control over the pool is lost due to thermal damage, and this material leaks into the environment. Sucrose (Fig. 4*b*) or sodium chloride (Fig. 5*b*), at 60°, apparently caused a more rapid degradation of RNA, although a longer period elapses before there is any appreciable loss of material from the metabolic pool. After 1 hr. at 60°, the greatest loss of RNA-like material occurs from cells held in sucrose, which confirms previous findings (1, 3); the loss from cells in water and saline appears to be of a similar, but lower, order.

A comparison has been made of the loss of viability of cells stored in water, 1 *M* sucrose or 0.5 *M* sodium chloride at 50 or 60°. The results are present in Fig. 6. A statistical analysis of the results was made, using a common regression line calculation, to test if the three regressions could be represented by a common line. During storage at 50° (Fig. 6*a*) sodium chloride had a significant protective influence on the loss of viability of the *Staph. aureus* suspension, but there was no significant difference in loss of viability of cells held in water or sucrose. During storage at 60° (Fig. 6*b*), the three regression lines could be represented by one common line, and any differences are thus not significant.

As evidenced by a rise in the content of RNA-like material in the metabolic pool, RNA degradation obviously occurs in heated *Staph. aureus* cells. The rate of degradation increases with temperature, and occurs before leakage takes place (particularly at 60°). Such a degradation may be responsible for thermally-induced death in this strain (1, 3) and in other strains of *Staph. aureus* (6, 7). It is, however, difficult to link this degradation with loss of viability of *Staph. aureus* held in different suspending media (Fig. 6). Moreover, in assessing the primary site of damage in heated cells, other changes in the organism must be considered, including changes in appearance under the electron microscope, in size, and in light-scattering properties (4). Thus, whereas RNA degradation may be the cause of death in heated cells (3, 6, 7) it is apparent that further research is needed to substantiate this contention.

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Renal Effects of a Vasodilator: 2-[*N*-methyl-piperidyl-(4)]-3-amino-5-(4′-pyridyl)-pyrazole HCl (Ciba 31-531 Ba)

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Abstract □ Acetylcholine (Ach), when infused directly into the renal artery of a kidney, causes unilateral vasodilatation and increased salt excretion. Ciba 31-531 Ba, a nonspecific vasodilator, was infused both directly into the renal artery and given systemically, and the effects were compared to Ach. Infusion of Ach at 0.1 μg./kg./min. into the left renal artery (LRA) of an anesthetized dog effected a unilaterally increased salt excretion and increased effective renal plasma flow (ERPF) and urine volume, but no demonstrable change in glomerular filtration rate (GFR) or blood pressure. Infusion of Ciba 31-531 Ba (LRA) at 0.1 mg./kg./min. resulted in a similar unilateral saluresis of approximately 50% increase over Ach with an increase in ERPF and urine volume but no change in GFR or blood pressure. The right kidney served as an internal control. When Ach and Ciba 31-531 Ba were given systemically, at higher doses there was a drop in blood pressure, decreased solute excretion, and decreased ERPF and GFR for both drugs. The effects of Ach were reversed with atropine. The effects of Ciba 31-531 Ba were not reversed by atropine or propranolol. The authors believe that the direct renal effects of these drugs are the results of direct tubular effects associated in some way with hemodynamic alterations.

Keyphrases □ 2-[*N*-methyl-piperidyl-(4)]-3-amino-5-(4′-pyridyl)-pyrazole HCl (Ciba 31-531 Ba)—renal effects □ Renal infusion—Ciba 31-531 Ba □ Acetylcholine, Ciba 31-531 Ba renal effects—comparison □ IV infusion, Ciba 31-531 Ba—renal effects

A new vasodilator, recently synthesized by Ciba (1), given systemically appeared to have contradictory effects on the kidney (2). In some cases it acted as a vasodilator and increased renal blood flow. In other experiments the various systemic effects appeared to predominate and no change or decrease in renal blood flow was observed.

Ciba compound 31-531 Ba, 2-[*N*-methyl-piperidyl-(4)]-3-amino-5-(4′-pyridyl)-pyrazole HCl, antagonized the effects of epinephrine, norepinephrine, angiotensin II-amide, and histamine on smooth muscle of the guinea pig and the cat. It did not antagonize the effects of $BaCl_2$, acetylcholine, or bradykinin (1).

When compared to papaverine, nitroglycerin, and isoproterenol in an open-chest cat preparation where it is possible to measure both systemic arterial and venous pressure, isoproterenol and Ciba 31-531 Ba administration caused both arterial and venous pressure to fall.

The direct renal effects of adrenergic and cholinergic agents have recently been reported in the literature (3). It was felt that a study of this rather nonspecific muscle-relaxing drug on renal function as compared to acetylcholine would be of interest. The following experiments were designed to study the effect on sodium excretion and renal hemodynamics when Ciba 31-531 Ba is given directly into the renal artery and when given systemically.

MATERIALS AND METHODS

Eight mongrel dogs were anesthetized with intravenous injections of pentobarbital sodium, 30 mg./kg. Both ureters were cannulated through an abdominal midline incision, and the cannulae were positioned approximately 1.27 cm. (0.5 in.) below the ureteral pelvic junction. A femoral vein and artery were cannulated and the arterial cannula was connected to an E & M linear transducer (E & M Co., Inc.) with a three-way stopcock for recording blood pressure with an E & M polygraph. Arterial blood samples were obtained through the three-way stopcock. The sustaining solution containing 1.8 mg./ml. of creatinine, 0.5 mg./ml. of *p*-aminohippurate (PAH) in normal saline was infused at a rate of 5 ml./min. through the venous system by means of a dual-syringe constant-flow pump. After exposing the left renal artery by the retroperitoneal approach, a 27-gauge hypodermic needle, attached to No. 10 polyethylene tubing, was placed into the left renal artery in the direction of blood flow. Through this renal arterial system, a solution of isotonic sodium chloride was continuously infused at a rate of 0.1 ml./min. Solutions of drugs were also infused at the same rate through the system by changing the renal arterial infusate to one containing test drugs dissolved in normal saline. One to two hours were allowed for equilibration and then collections of 10-min. urine samples from each kidney were begun. Blood samples, drawn every 20 min., were heparinized, centrifuged, and the plasma immediately removed. At