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# Urinary Procoagulant Excretion in Experimental Renal Disease* (32643)

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Human urine contains a powerful procoagulant (1). It converts prothrombin into thrombin in the presence of factor V, lipid, and calcium (2), a reaction which is the basis for its quantitative assay (3). The procoagulant content of the urine in patients with thrombosis, embolism, myocardial infarction, and hemorrhagic diathesis is the same as that of healthy subjects. There is, however, a statistically highly significant ($p < .001$) decrease of the urinary procoagulant in parenchymatous kidney diseases (4). Numerous patients with nephrotic syndrome and acute tubular necrosis did not excrete it at all. The present investigations were designed to eliminate urinary procoagulant excretion in animals and thereby to obtain information on the origin of the procoagulant.

*Material and Methods.* Quantitative determination of the procoagulant content of the urine: A modificaiton of a previously described method (3); 0.2 ml urine are required.

Hematuria was graded according to the number of erythrocytes in urine sediment per high power field. —: 0 erythrocytes; +: 1–3; ++: 4–10; +++: 10+.

Glycosuria was estimated with Combistixs (Ames Corp., Elkhart, Indiana).

Protein content of the urine was estimated with Exton's reagent (5) and quantitated with Esbach's procedure (5).

*Urine collection.* Urine was collected 8:00 a.m. and immediately dialyzed in the cold. In the phlorizin studies, urine was obtained by an indwelling catheter.

Male albino rabbits, 2–3 kg were purchased locally. Male white Sprague-Dawley rats, approximately 200 gm, were obtained from the Simons Lab., Gilroy, California. The animals were fed Purina Chow. They were housed in stainless steel metabolic cages constructed to prevent contamination of urine with feces. After urine collection, all cages were thoroughly cleaned daily.

*Nephrotoxic agents. (a) Duck antirabbit-kidney serum* (Antibody Incorp. Davis, California). The serum was inactivated for 30 min at 56°C and then adsorbed for 30 min at 0°C with 1/10 vol. washed rabbit erythrocytes. One single dose of 2 ml was given

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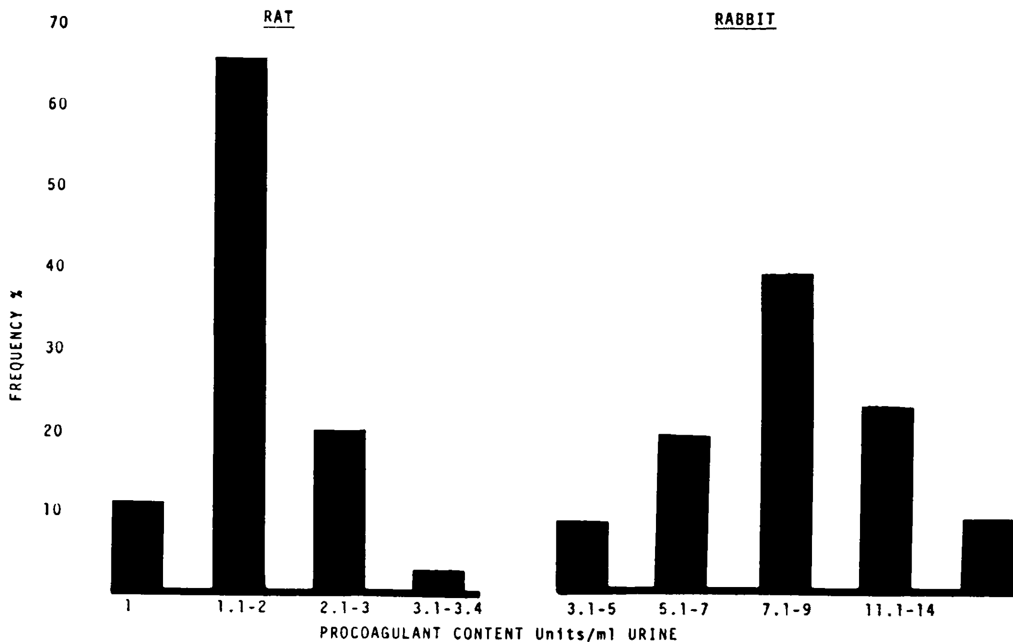


FIG. 1. Frequency distribution of procoagulant content in rabbit and rat urine.

intravenously. *(b) Aminonucleoside.* Puromycin (6-dimethylamino-9-[3-desoxy-3-(*p*-methoxy-L-phenylanylamino)-$\beta$-D-ribofuranosyl]-$\beta$-purine); (Nutritional Biochemicals Corp., Cleveland, Ohio), 1.5 mg/100 gm in a 0.5% solution in distilled water was injected subcutaneously daily for 3 days into rats. *(c) Phlorizin* (Aldrich Chemical Corp., Milwaukee, Wisconsin) was made up to 20% solution in 10% $NaHCO_3$. One single intravenous dose of 200 mg/kg was given to rabbits. *(d) Albumin.* Bovine albumin (fraction V) in a 35% sterile solution (Nutrit. Biochem. Corp.) was diluted to 15% in 0.45% NaCl; 1.5 gm in 10 ml was given daily intraperitoneally to rats for 3 days. *(e) Mercury chloride.* (Matheson Coleman & Bell) 3 mg/kg of a 0.7% solution in distilled water was injected intravenously once into rabbits. *(f) Histochemistry.* Hale's method for acid mucopolysaccharide (6) was used.

*Results. (i) Procoagulant content of the urine of normal male rabbits and rats. (a) Rabbits.* Fifty-six determinations in 30 healthy rabbits revealed an average procoagulant content of 8.23 units/ml of urine (SD 2.34).

*(b) Rats.* The values of 35 determinations in 16 rats were 1.85 units/ml (SD 0.54). The frequency distribution of these values is shown in Fig. 1.

*(ii) Masugi nephritis in rabbits* (7). Masugi nephritis was induced with the rabbit antikidney serum in 5 rabbits. In all animals, moderate oliguria and loss of appetite developed after 24 hours followed within 5–8 days by a marked proteinuria and hematuria. The first day after antiserum injection, the urinary procoagulant dropped (av. —50%) and remained at this level until proteinuria and hematuria developed at which time the values returned to normal. During actual nephritis, the procoagulant values were essentially normal and remained so during the further course of the disease (Fig. 2).

*(iii) Aminonucleoside-induced nephrosis in rats* (8). Experimental nephrosis was induced in 9 rats. Mild proteinuria (50 mg protein loss per day) developed 1 week after the first injection, became more severe (500 mg) during the next 1–2 weeks, and then disappeared. The week following the injection, the procoagulant content of the urine gradually dropped to zero, was absent during the phase of maximum proteinuria, and returned to normal as the proteinuria diminished (Fig.

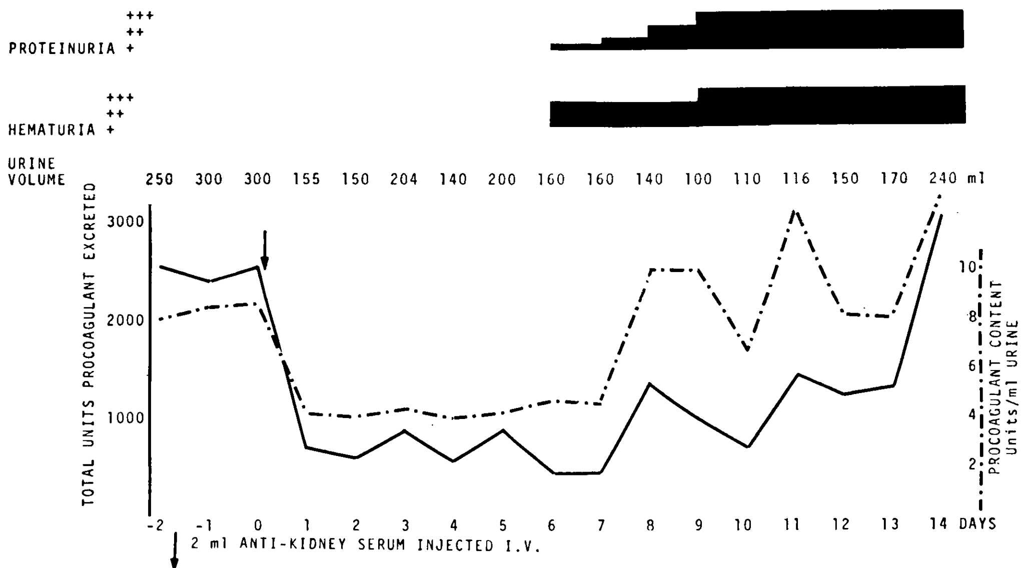


FIG. 2. Trend of total procoagulant excretion and content ml/urine in Masugi nephritis in a rabbit.

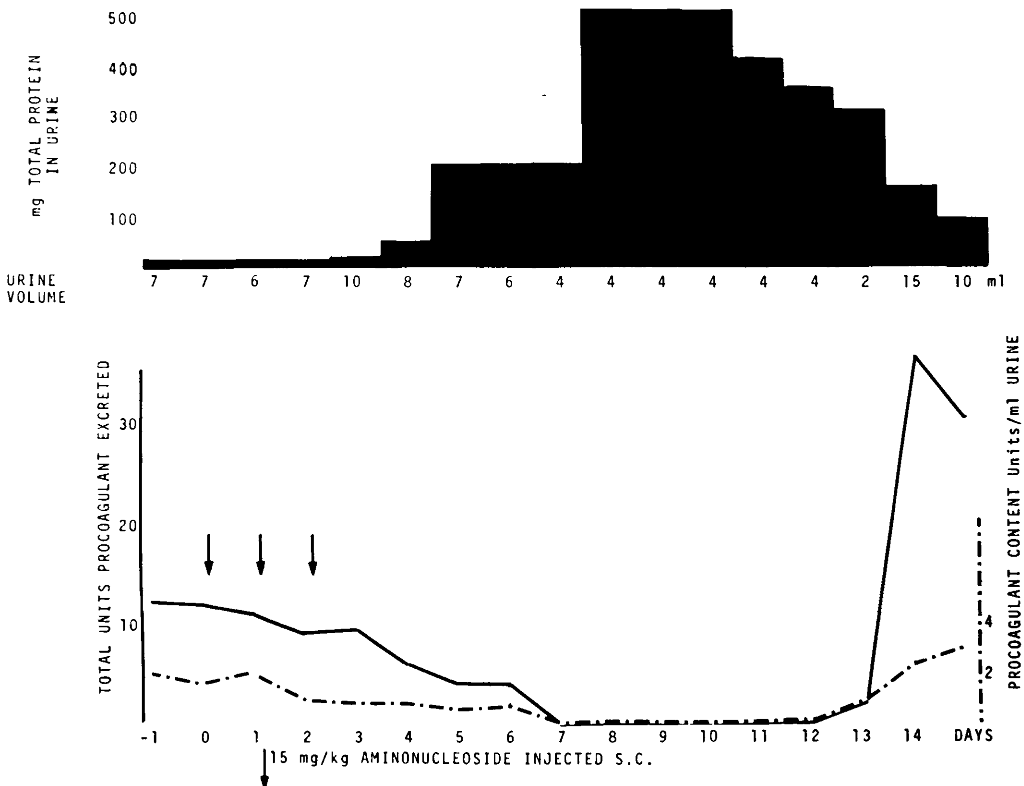


FIG. 3. Trend of total procoagulant excretion and content ml/urine in aminonucleoside nephrosis in a rat.

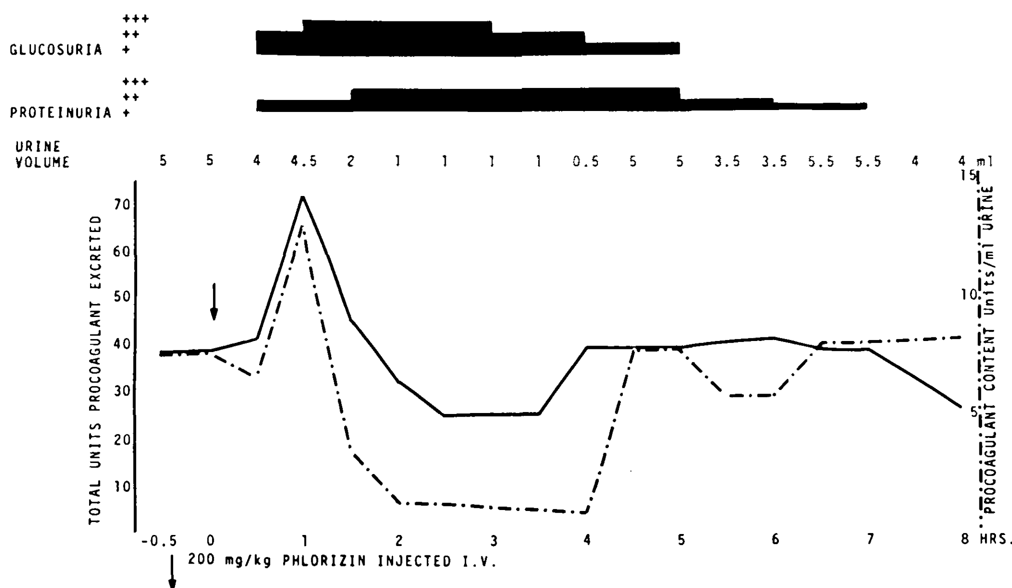


FIG. 4. Trend of total procoagulant excretion and content ml/urine in phlorizin-induced tubular blockage in a rabbit.

3). Two rats received four additional injections resulting in absence of the procoagulant together with a persistent proteinuria for a 3-months' observation period.

*(iv) Blockage of tubular reabsorption of glucose* (9). Tubular reabsorption of glucose was blocked by phlorizin in 4 rabbits. Thirty minutes after injection, glucosuria and proteinuria appeared with no change in procoagulant content of the urine. The procoagulant started to rise (av. +172%) in about 1 hour together with an increase of glucosuria. At 2–3 hours, a moderate drop (av. —39%) of the procoagulant occurred with return to normal at about 4 hours at which time a mild proteinuria and glucosuria were still present (Fig. 4).

*(v) Albumin-induced proteinuria* (10). Reversible proteinuria was induced by albumin overload in 8 rats. Marked proteinuria was evident the day after the first bovine albumin injection and generally increased during the injection series. The urinary procoagulant rose severalfold (av.+508%) during the first 2 days and decreased subsequently to zero as the protein excretion increased. The initial rise occurred in seven out of eight animals, but the temporary loss of procoagulant excretion occurred in all of them. After termination of albumin loading, the proteinuria tapered off and the procoagulant excretion was rapidly resumed (Fig. 5).

*(vi) Mercury nephrosis* (11). Toxic nephrosis was produced in 10 rabbits with iv $HgCl_2$ injection. There was a marked rise (av. +301%) in seven out of ten animals of the procoagulant content of the urine after approx. 12 hours. Diarrhea, loss of appetite, proteinuria and glucosuria developed within 24 hours. The procoagulant increase was followed by a rapid drop to zero for 1 to several days with subsequent return to normal 1–3 days preceding disappearance of glucosuria and proteinuria (Fig. 6).

*(vii) Histochemical observations.* Staining of kidney slices for acid mucopolysaccharides was carried out in untreated rabbits, during the transitory increase of the procoagulant content of the urine, and at zero excretion. Normal rabbit kidneys occasionally showed a faint positive Hale stain of the tubular epithelium. Hyaline droplets in the tubular epithelium in albumin-induced proteinuria and in necrotic material in the tubular lumen in $HgCl_2$ nephrosis gave a strongly positive Hale stain. At zero excretion, no more Hale posi-

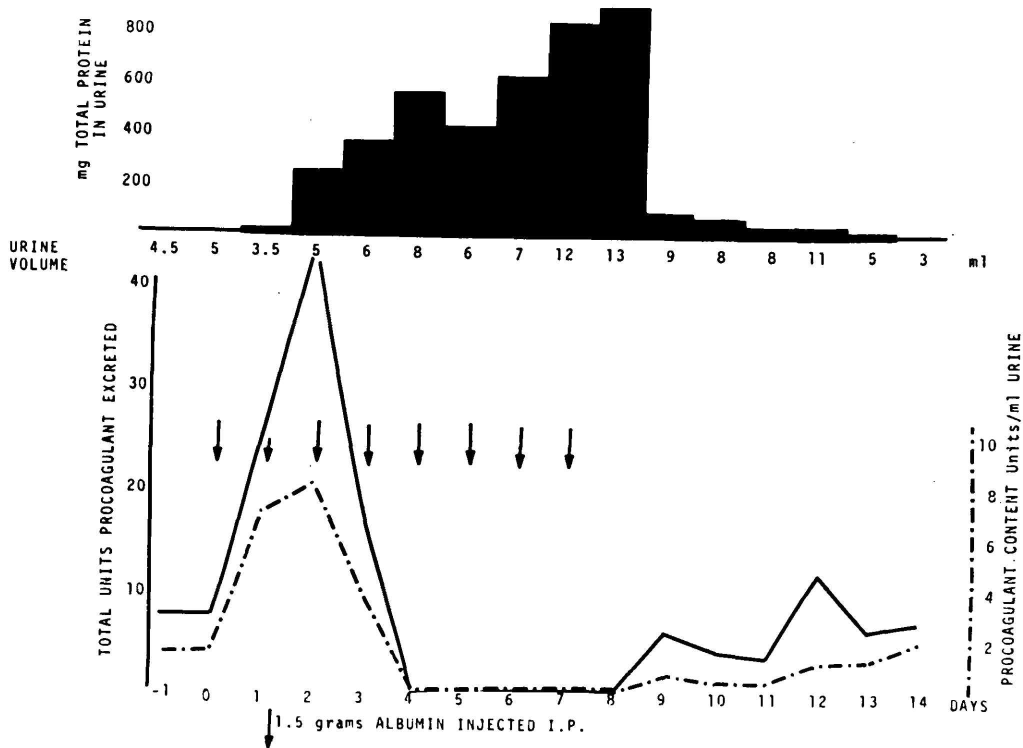


FIG. 5. Trend of total procoagulant excretion and content ml/urine in albumin-induced proteinuria in a rat.

tive substances could be demonstrated in the tubules.

*Discussion.* Beyond the thrombin generating capacity which served to identify it, little is known about the properties of urinary procoagulant. The material has a molecular weight above 200,000 (exclusion by Sephadex 200) (3). That a purified, though still heterogenous, preparation may be stained with Hale's stain indicates the presence of mucopolysaccharides (3).

Its presence in urine posed the problem of whether it is present in blood and excreted by the kidney, or is a product of the kidney or lower urinary tract. Its disappearance from urine, not only from patients with renal failure but also from urine of patients with the nephrotic syndrome without azotemia, suggested that it is produced in the kidney. Furthermore, since the two clinical conditions in which it disappeared from the urine involve the proximal tubule (the nephrotic syndrome and acute tubular necrosis), the proximal tubule became suspect as the site for procoagulant production. This clinical association is extended to experimental animals by the present study. Production of heavy proteinuria by administration of aminonucleoside or heterologous albumin resulted in disappearance of urinary procoagulant. This effect was also produced by $HgCl_2$ damage to the proximal tubule. It was thus possible to eliminate urinary procoagulant excretion by experimental procedures aimed at the renal tubule.

However, the factors governing urinary procoagulant excretion appear to be quite complex. The decrease of urinary procoagulant excretion in the phlorizin, albumin, and $HgCl_2$ treated animals was preceded by an increase in its excretion. In Masugi nephritis, the decreased procoagulant excretion preceded the proteinuria and hematuria and indeed had returned to normal when this urinary evidence of glomerular damage was maximal. This normalcy of urinary procoagulant excretion at the time of maximal glomerular

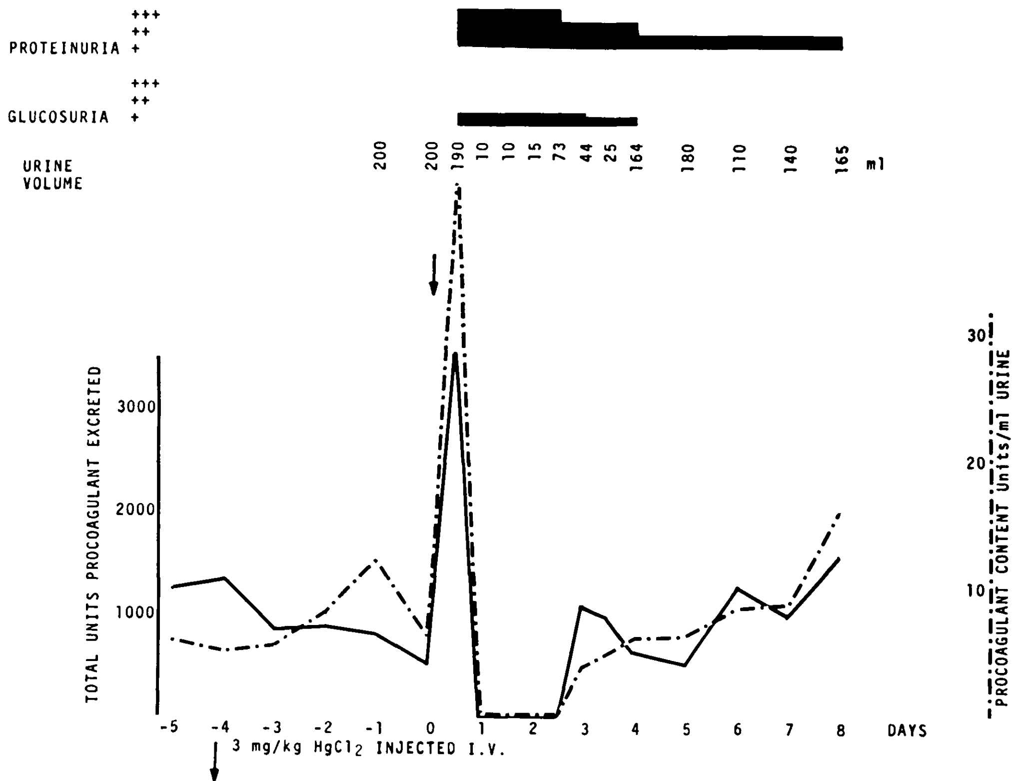


FIG. 6. Trend of total procoagulant excretion and content ml/urine in mercury-induced nephrosis in a rat.

damage focused attention on the tubule as the source of procoagulant.

Histochemical evidence that the renal tubule was the source of procoagulant was sought with the Hale stain for mucopolysaccharide which does react with purified, though heterogenous, procoagulant. Although tubules of normal animals were faintly Hale positive, there was a correlation between the intensity of the staining and the procoagulant excretion in the $HgCl_2$ treated animals during the period of increased excretion as well as the period of decreased excretion.

Thus, the fact that a variety of experimental renal disorders influence urinary procoagulant excretion suggests that the kidney is the source of urinary procoagulant. Identification of mucopolysaccharide in the renal tubule suggests the tubule as the immediate source of urinary procoagulant.

The rate of release of procoagulant into the urine is likely to be influenced by many variables including rate of accumulation in the tubule cell and the structural and functional integrity of the tubule cell. The pattern of change in urinary procoagulant excretion observed with each of the differing experimental procedures, as well as the differences in the effect of each type of procedure, suggests that many factors influence the rate at which the tubule releases urinary procoagulant.

*Summary.* Nephrotoxic agents temporarily alter the excretion of a procoagulant normally present in the urine. Agents producing tubular necrosis or inducing proteinuria without severe glomerular damage have the most pronounced effect.

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# Inhibition of Hemolytic Activity of El Tor *Vibrio* by Antibiotics Which Interfere with Protein Synthesis* (32644)

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The inhibition of protein synthesis by antibiotics such as tetracycline, chloramphenicol or erythromycin has been found to be quite selective. Chloramphenicol and erythromycin, for example, suppress protein synthesis and the adaptive formation of beta-galactosidase; inhibition of the induction of this enzyme in *Escherichia coli* occurs at concentrations of antibiotic that permit exponential growth (1, 2). Oxytetracycline has been noted to interfere preferentially with the induction of penicillinase in *Staphylococcus aureus* (3).

The purpose of the study reported in this paper was to investigate the possibility of selective inhibition of the hemolytic activity of the El Tor strain of *Vibrio cholera* by antibiotics that alter protein synthesis. The data obtained indicate that, under certain conditions, inhibition of hemolysis may follow exposure to some antimicrobial agents without the development of obvious changes in the growth characteristics of the organism.

*Materials and Methods.* The El Tor strain of *V. cholera* used in these studies was obtained from American Type Culture Collection and was maintained in the laboratory by repeated passages on 5% horse blood agar.

The antibiotics employed included conventional commercial preparations of penicillin, streptomycin, polymyxin, tetracycline and oleandomycin, paromomycin, chloramphenicol, erythromycin, cephalothin, kanamycin and colistin. Varying concentrations of each compound were prepared in heart infusion broth. These were mixed with a $10^{-3}$ dilution of an 18-hour broth culture of *V. cholera* in equal volume and incubated at 37°C for different periods of time. Deep and surface subcultures from these mixtures were made on 5% horse blood agar and incubated at 37°C overnight, at which time the presence or absence of beta-hemolysis was noted.

*Results. Inhibition of hemolysis by single antibiotics.* Subcultures of *V. cholera* from broth containing tetracycline, chloramphenicol, oleandomycin, erythromycin and paromomycin failed to produce hemolysis. The degree of inhibition of hemolytic activity appeared to be related to the concentration of the drug and the length of time over which the organism was exposed to it. As shown in Table I, inhibition of hemolysis was demonstrable only when the level of the antibiotics exceeded their minimal inhibitory concentration (MIC). Tetracycline and chloramphenicol were effective at 5–10 times their MIC, whereas the other antimicrobiol agents were active only at 10 times their MIC.

That the duration of exposure of the organism to a drug was of importance in suppressing its hemolytic activity is illustrated by the data presented in Table II. Production of

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