TABLE II.
Burn Shock Mortality in Treated and Control Dogs.

Treatment	No. of animals	% mortality 24 hr	Avg hr survival
Benadryl i.m.	28	89	11.5
Controls—Saline i.m.	26	85	12.9
Benadryl + saline i.v.	11	64	19.4
Control—Saline i.v.	11	73	14.4

therapy, although there may be some suggestion that saline infusion plus this drug aided survival. Hematocrit, and blood and serum specific gravity changes did not vary consistently between treated and control groups. Blood pressure readings in the treated group tended to be slightly above those of the controls throughout the survival period, but this did not appear to be of significant magnitude.

Discussion. The results above indicate that the 2 antihistaminics used were without benefit, or somewhat detrimental, to animals in burn and tourniquet shock. Page and Green(7) found that large doses of Benadryl caused vascular refractoriness in the dog, with the result that larger than normal amounts of saline could be infused into the animal. This was ascribed to increased capacity of the vascular bed, or to increased permeability of the capillaries. Our results in the shocked dog do not suggest this, since the changes in blood pressure and hemoconcentration were not consistently different in the control and treated animals. Possibly the larger doses of Benadryl used by Page and Green account for the refractory state of the vascular bed in their animals.

Summary. The administration of Benadryl or Thephorin to rats subjected to tourniquet shock tended to increase mortality if given in relatively large doses. Dogs in burn shock treated with Benadryl did not differ significantly from untreated controls with respect to mortality, blood pressure, hematocrit, and blood and serum specific gravity.

7. Page, I., and Green, A., *Am. J. Physiol.*, 1949, v156, 405.

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A Rapid Method for Determination of the Aureomycin Concentration of the Blood. (17824)

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The need for a speedy report of the blood concentration is particularly urgent with aureomycin therapy. Nausea, vomiting and diarrhea are not uncommon with this drug, and it is often difficult to decide whether or not the administered drug has been adsorbed and to what extent. This need is further emphasized by the increasing use of such adjuvants as aluminum hydroxide gels to counteract the toxic symptoms. Waisbren and Hueckel(1) have shown that when aluminum hydroxide gel is administered with aureomycin, a lowered level of aureomycin in the blood results. A method of assay which can be completed and reported within 5 hours after the specimen is received in the laboratory, no matter what time of day or night, is the subject of this report. The procedure is a tube dilution method, employing *Proteus vulgaris* OX-19 as the standard organism and a special urea-phenol red broth as the medium. The principle of the test is based upon the bacteriostatic action of the antibiotic on the test strain and the inhibition of the rapid

1. Waisbren, B. A., and Hueckel, J. S., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 73.

TABLE I.

					ml					Controls	
Aureomycin Standard											
Aureomycin in Normal Human Serum (2 μg/ml)				.5	.4	.3	NHS*	0	1	0	
Urea Medium				.5	.6	.7		1	0	1	
Standard Inoculum				1.	1.	1.		1	1	0	
Serum Sample.											
Serum	1.	.8	.6	.5	.4	.35	.3	.25	.2	.15	.1
Urea Medium	0	.2	.4	.5	.6	.65	.7	.75	.8	.85	.9
Standard Inoculum	1.	1.	1.	1.	1.	1.	1.	1.	1.	1.	1.

* Normal Human Serum.

reaction and color change when it splits urea to ammonia. No other organisms are capable of producing the color change within the time limit of the test, thus obviating the need for sterile technic. Since the incubation period at 37°C is approximately 4 hours, deterioration of aureomycin is minimal. Finally, the endpoints are indicated by color changes and are easily read.

Method. Blood for assay is collected in a dry, clean centrifuge tube and the serum is separated in the usual fashion. Care must be exercised to prevent hemolysis as the test is dependent upon the development of a red color. The test employs 2 ml amounts and is performed in unplugged 12 x 100 mm tubes. Clean, but not sterile glassware, pipettes, etc. are used. The medium described by Stuart, Van Stratum and Rustigan (2), modified by the addition of 1% Bacto-Peptone as added nutritive material in order to accelerate the growth of the test organism, is employed. It is prepared as follows: To 380 ml of distilled water are added 3.64 g KH_2PO_4, 3.8 g Na_2HPO_4, 8.0 g Urea C. P., 40 mg Yeast Extract Difco, 4.0 g Bacto-Peptone Difco, and 20 ml Phenol Red (0.02%). The pH is usually 6.5-6.6, and is adjusted by the addition of 10% NaOH to a final pH of 6.8, at which point the medium is colored deep amber. It is stored in the refrigerator without being sterilized. The amount necessary to perform one or a series of tests is withdrawn as needed. The American Type Culture Collection strain of *Proteus vulgaris* OX-19 is the standard organism. Plain fresh meat extract broth is inoculated with this organism and incubated for 4 hours at 37°C and then diluted 1:5 with the same broth. The diluted broth is distributed into small tubes in approximately one milliliter amounts and stored in this manner in the refrigerator as individual stock cultures. These can be kept and used for 2 weeks. When indicated, a tube is removed and used to prepare the standard inoculum, which is a 1:100 dilution of the stock culture in the urea medium. Preliminary incubation of the standard inoculum is unnecessary, hence the test can be started at any hour of the day or night.

The aureomycin standard, 2.0 μg of aureomycin per ml in normal human serum, is freshly prepared just prior to the assay from a concentrated standard maintained in the freezing compartment of the refrigerator. Pooled excess serum from specimens sent for serological examination is readily available in the laboratory and can be used for the preparation of the aureomycin standard and for the controls.

Two parallel series of tubes are set up as shown in Table I.

Both series of tubes are incubated at 37°C for 4 hours at which time the inoculated controls usually have a definite red color. Occasionally, 4½-5 hours are required to complete the reaction. The endpoint, indicating complete inhibition of the test organism by the antibiotic, is the first tube in each series in which the amber color fails to show any change to red. The serum level is calculated by dividing the amount of standard antibiotic required to inhibit the standard inoculum by the amount of serum required to inhibit the same inoculum. If inhibition takes

2. Stuart, C. A., Van Stratum, E., and Rustigan, R., *J. Bact.*, 1945, v49, 437.

place throughout the entire serum series, the serum is diluted 1:10 in the urea medium and the test is then repeated with diluted serum. The level is calculated in the same manner, multiplied by the dilution factor.

Experimental results. It was found that the standard inoculum was consistently inhibited by 0.8 μg of the standard aureomycin. Thus the minimum assayable concentration of aureomycin in the serum is 0.8 μg per ml of serum. Although this is not as sensitive as methods in current use, it is adequate for clinical purposes, since the minimum detectable level is well within the range of blood concentrations regularly found with the doses commonly employed.

To test the accuracy of this procedure, 36 samples of normal human serum were fortified with known concentrations of aureomycin just prior to testing and then assayed. The results of the assay proved accurate in 28 out of 36 tests, or 77.8%. In the remaining tests there was a discrepancy between the prepared and calculated values, but in no instance was there any error greater than one tube, except in one, which was two tubes under. Considering the experimental error arising from the preparation of the test specimen as well as the performance of the assay, a high correlation was found between the concentration of the prepared specimens and the result of assay.

Summary. A rapid method for the determination of the aureomycin concentration of the blood is described. Sterile specimens and sterile technic are not required. The procedure is based upon the inhibition of the test organism, *Proteus vulgaris* OX-19 by the antibiotic, thereby preventing the reaction and color change produced in the urea-phenol red medium when urea is split to ammonia by the test strain. The minimum assayable level is 0.8 μg per ml of serum. A high degree of correlation was found between the actual value and the results of assays with 36 prepared specimens.

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Characterization and Separation of an Inhibitor of Viral Hemagglutination Present in Urine. (17825)

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Hemagglutination caused by influenza, mumps and Newcastle disease viruses is inhibited by a number of biological materials which possess such activity in varying degree(1-3). The various inhibitors have certain properties in common: when tested with appropriately heated viruses, they are active in high titer, but their activity is destroyed by the unheated viruses; it is also destroyed by cholera vibrio extract, crystalline trypsin, or periodic acid. The inhibitors are substances of high molecular weight and are thought to be mucopolysaccharides or mucoproteins. The interaction between inhibitors and viruses has been studied in considerable detail by numerous workers(4-7), but purification of the inhibitors has met with difficulties which have not been completely overcome(7-14). Among the most

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5. Svedmyr, A., *Brit. J. Exp. Path.*, 1949, v30, 237, 248, 254.
6. Lanni, F., and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1948, v68, 442.
7. Hardy, P. H., Jr., and Horsfall, F. L., Jr., *J. Exp. Med.*, 1948, v88, 463.