TABLE I. Hematologic Data on Patients with Pernicious Anemia in Relapse Treated with 1 and 2 μg Vitamin B_{12} Daily.

B_{12} dose, μg/day	Patient	Initial Hb, g	Final Hb, g	Initial R.B.C. $\times 10^6$	Final R.B.C. $\times 10^6$	Retic. % peak/day	Days of observation	Remarks	Days
1	G	8	14	1.89	4.37	8.1/7	53	Blood count stationary	25
	P	5	10	1.46	3.48	10.4/10	63	" " "	23
	M	6	15	1.40	4.67	21.0/8	108		
	V.H.	7.5	12	2.43	3.66	No*	93	" " "	59
2	S	8	15	2.30	5.10	16.4/6	132	Normal blood count in	48
	Mc.	6	14	1.85	4.75	20.6/7	83	" " " "	69
	Mi.	5.5	13.8	1.25	4.85	40.9/8	250	" " " "	47
	F	5.5	12	1.50	4.60	33.0/9	48	" " " "	48

* No retic. response.

cyte response was optimal in 3 instances and submaximal in the fourth case. Normal hematologic levels were attained in 47 to 69 days. The clinical response as regards appetite, gain in weight, strength and improvement in well being, were the same as those observed with adequate doses of liver extract or folic acid. All four cases showed moderate to advanced neurologic changes prior to treatment. No improvement, other than disappearance of numbness and tingling in one patient was observed.

The authors have under treatment 2 groups of pernicious anemia patients in remission who are receiving 14 μg of crystalline vit. B_{12} intramuscularly every 7 or 14 days. It is too early to compare these data with those presented by Meacham *et al.*(5).

Summary. (1) Four patients with pernicious anemia in relapse were treated with 1 μg of crystalline vit. B_{12}, intramuscularly, daily. Three cases showed submaximal reticulocyte responses and failed to reach normal blood levels in 53 to 93 days. Clinical improvement was observed in all instances. (2) Four other patients with pernicious anemia in relapse, treated with 2 μg of crystalline vit. B_{12}, intramuscularly, daily, showed satisfactory clinical improvement, optimal reticulocyte responses, and normal blood levels in 48 to 69 days. Symptoms of neurologic disease disappeared in one person but persistent signs were observed in all 4 cases.

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Inhibition of Phagocytosis by Aureomycin. (18201)

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Aureomycin is a widely used antibiotic in the therapy and control of many bacterial, viral and rickettsial diseases(1). It is apparently tolerated in large doses in human patients as well as in experimental animals, and aside from minor gastro-intestinal disturbances and rare allergic reactions, few untoward signs of toxic manifestations have been observed(2,3). The doses administered clinically range from 50 mg/kg/day to 500 mg/kg/day orally(4,5), resulting in blood levels of .15 μg to 20 μg per ml. Lepine and co-workers(6) reported that aureomycin as

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well as chloramphenicol (Chloromycetin) was toxic to developing cells as shown by their complete inhibition of the growth of cells in tissue culture by these antibiotics. Because of our interest in the role of the phagocytic system in the recovery from infections treated with antibiotics, an attempt to investigate the effect of aureomycin on the *in vitro* phagocytosis of bacterial cells by the polymorphonuclear leucocytes of normal human blood was initiated.

Materials and methods. The crystalline aureomycin used in this work was purchased from Lederle Laboratories as the parenteral hydrochloride salt (lot No. 7-9837). The solutions of the drug were made in M/15 phosphate buffer at pH 7.2 and used within one hour after they were made. The blood utilized was drawn from the median cephalic vein at the antecubital fossa of a normal subject. It was immediately placed in a flask containing enough saline solution of heparin (20 mg/ml) to give a final concentration of 2 mg/ml blood. The heparinized blood was used within 15 minutes after its collection. A culture of *Micrococcus pyogenes var. albus* previously used in similar studies was selected (7) because it is readily phagocytized by normal human leucocytes, and forms a smooth suspension in saline which can easily be standardized. The organisms were grown on plain nutrient agar slants for 12 hours at 37°C, washed off the slants with saline and a suspension corresponding to nephelometer standard No. 1 was made (approximately 300 million organisms per ml).

The phagocytic tests were set up as follows: 0.3 ml of M/15 phosphate buffer was added to each tube (9 mm x 75 mm), except to the control tubes containing no aureomycin which received 1.3 ml of the same buffer. One ml of aureomycin solution in the proper concentrations was added to all tubes except the controls, and following this 0.5 ml of the freshly drawn heparinized blood was pipetted into each tube. Finally, 0.2 ml of the standardized suspension of organisms was added to all the tubes. The whole procedure never took more than half an hour to be completed, and the addition of the blood and suspension of organisms was done within 5 minutes. The final volume in each tube was 2 ml. The tubes were then stoppered with corks which had been kept in distilled water for a few days to extract any substances which might have interfered with phagocytosis. The tubes were then placed in a rotating disk (15 rpm) completely submerged in a water bath at 37°C as previously described by Hanson(8). They were rotated for 15 minutes and as soon as possible after this rotation period elapsed 4 smears were made on clean slides from each tube, starting with the tube containing the highest concentration of aureomycin. The time elapsing between the end of the rotation period and the completion of making the smears was never longer than 10 minutes. To be sure that this time did not affect the number of cells showing phagocytosis, another smear was made of the first tube after it had stood for half an hour at room temperature without rotation. No further change in phagocytosis was ever noted in this tube. pH readings before and after the experimental procedure remained at 7.2. The smears were stained with the methylene blue method of Welch and Hunter(9). Four hundred polymorphonuclear leucocytes were counted under the oil-immersion lens of the microscope (100 from each of 4 slides), and the ones showing one or more ingested micrococci were recorded. In some series Wright's stain was used, but this was found to be less satisfactory than the methylene blue stain of Welch and Hunter. These experiments were always run in duplicate and were repeated several times with similar results.

Results. The results of these tests are tabulated in Table I, where the statistical

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TABLE I. Effect of Aureomycin on Phagocytosis.

Tube No.	Conc. of aureomycin μg/ml	% phagocytosis in 4 separate slides				Avg % of phagocytosis	Total No. of cells phagocytizing of 400 cells counted	Statistical analysis		
		1	2	3	4			X^{2*}	P*	
1	1000	10	6	8	8	8	32	190.62	<.01	
2	100	22	23	23	24	23	92	85.84	<.01	
3	10	30	33	31	35	32.2	129	35.18	<.01	
4	5	30	30	36	34	32.5	130	34.31	<.01	
5	1	35	35	41	38	37.2	149	20.02	<.01	
6	0.1	45	43	45	43	44	176	6.48	<.02	>.01
7	0.01	49	43	47	44	45.7	183	4.24	<.05	>.02
8	0.001	45	46	52	50	48.2	193	1.80	<.20	>.10
9	0.0001	46	53	48	50	49.2	197	1.05	<.50	>.30
10	0.00001	48	47	52	52	49.7	199	0.84	<.50	>.30
11	0.0	55	54	52	52	53.2	213			

* The chi square (X^2) values were calculated by the 4-fold table method, as described in "Statistical Methods" by G. W. Snedecor. The Iowa State College Press, Ames, Iowa (4th edition), page 199. The probability number (P) was calculated from a table given in the same book on page 190. Only values of P less than 0.05 are here considered to indicate a statistically significant difference.

analysis of the difference between the number of leucocytes showing phagocytosis in the control tubes and the number in each of the tubes containing aureomycin is also given. It can be readily seen from this table that aureomycin had a pronounced inhibitory effect on phagocytosis. The percentage of phagocytosis in the tubes containing 1000 μg of aureomycin per ml was 8, while the control tubes containing no antibiotic gave an average of 53.2%. This inhibition was less pronounced, but still statistically significant, as the aureomycin concentration was decreased to 0.01 μg/ml ($X^2 = 4.24$, and $P < .05 > .02$). This inhibitory effect of aureomycin was gradually decreased as the concentration of the drug was reduced, but even in the lowest concentration used (.00001 μg/ml) the percent of phagocytosis was less than in the control. These differences in the higher dilutions, however, are not statistically significant (Table I). The decrease in phagocytosis was marked in concentrations of aureomycin that are commonly found as therapeutic blood levels in human patients (1 to 10 μg/ml), and even below.

Discussion. The results of this work indicate that aureomycin in concentrations of 1000 μg/ml inhibited phagocytosis almost completely. This inhibition was also marked at concentrations below this level and at concentrations which are generally found as therapeutic blood levels in human patients (4,10-12). Phagocytosis was still inhibited to a significant degree when aureomycin was diluted to 0.01 μg/ml, which is far below the therapeutic blood levels which are generally recommended. Apparently the lowest concentration reported clinically is 0.15 μg/ml (4), and 20 μg/ml serum or higher are often obtained(4,11). Since there are many pathogenic organisms which are resistant to this antibiotic(13), the results reported here suggest the possibility that in those cases where the infections are due to aureomycin-resistant organisms the use of this drug may actually be harmful since aureomycin appears to interfere with phagocytosis.

Summary. Phagocytosis of *Micrococcus pyogenes var. albus* cells by polymorphonuclear leucocytes was markedly inhibited by aureomycin in a concentration of 1000 μg/ml *in vitro*. This inhibition, although less pro-

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nounced, was still significant in concentrations of the antibiotic which were considerably lower than the therapeutic blood levels ordinarily employed in clinical practice.

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Cytopathogenic Effect of Poliomyelitis Viruses *In vitro* on Human Embryonic Tissues.* (18202)

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We have previously described the cultivation of the Lansing and Brunhilde strains of poliomyelitis virus in suspended cell cultures of human non-nervous and nervous tissue (1,2). It was also noted that degenerative changes occurred in cells from cultures inoculated with the Lansing virus(1) that were more extensive than in tissues from uninoculated cultures. This phenomenon has been more extensively investigated. The most significant observations will be here summarized; a more detailed account will be published later.

Materials and methods. Suspended cell cultures. The procedures have been previously described(1,3). As routine 50 units of penicillin and 50 μg of streptomycin per cc have been included in the medium. *Plasma hanging drop cultures.* Fragments of tissue from suspended cell cultures were explanted in plasma hanging drop cultures. The medium in which each fragment was embedded consisted of 2 drops of heparinized fowl plasma, one drop of chick embryo extract and one drop of beef embryo extract. Usually 8 cultures were prepared in a petri dish(4). This method permits the study of large numbers of fragments with economy of time and effort. *Roller tube cultures.* The general method has been presented in detail(5). The medium used in these experiments consisted of 9 parts of the Hanks-Simms solution adopted as routine for the suspended cell cultures(1,3) and 1 part of beef embryo extract. Two cc of this mixture was employed in each culture. *pH determinations.* The pH of suspended cell cultures was determined colorimetrically by direct comparison of each culture with a set of standard buffer solutions. The latter were prepared in 25 cc Erlenmeyer flasks each containing 3 cc of solution and the same concentration of phenol red as the Hanks-Simms solution used as culture medium. *Sections of tissue fragments from suspended cell cultures.* At the end of an experiment, Zenker's solution without acetic acid was added to the fragments in each flask. After fixation the fragments from one set of cultures were combined, embedded and stained with hematoxylin and eosin. *Viruses.* The Lansing and Brunhilde strains of poliomyelitis virus have been used(1,2). *Antisera.* Specific monkey antiserum for the Brunhilde strain was obtained through the courtesy of Dr. Jonas Salk. Lansing immune serum was prepared in this laboratory by repeated intramuscular injection of a monkey with infected monkey cord. This serum diluted 1:1000 completely protected mice against 1-3 LD_{50} of virus, but failed to protect in a dilution of 1:5000.

Experimental. Changes in cell morphology

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