placed in a shaking machine in the incubator at 28°C. The activity of the culture filtrates was determined after 3, 5 and 7 days incubation by the cup method against a streptomycin standard (Table II).

The results show that the freshly isolated cultures were all active producers of streptomycin. That the antibiotic formed by the unknown strains of *S. griseus* was streptomycin or at least a streptomycin-like substance is illustrated by the similarity in the antibiotic spectra of the different preparations.

To illustrate further the effect of phage upon the unknown strains, the cultures were streaked upon agar plates. These were incubated for 24 hours, metal rings placed upon a portion of the area of growth of the organisms, and a few drops of actinophage placed inside each ring. The plates were incubated another 24 hours, the rings removed, and the plates examined. The action of the phage is illustrated in Fig. 1. The effect of the phage was exactly the same as that usually produced on the streptomycin-producing strains of *S. griseus*.

Summary. A method for the rapid isolation and identification of streptomycin-producing strains of *Streptomyces griseus* is described. The method consists in plating out various dilutions of material from natural substrates, using agar media to which 25 μg/ml streptomycin has been added. Colonies of actinomycetes developing on the plates are picked and transferred to agar slants. When the cultures have developed, they are used to make agar streaks on plates. The plates are incubated for 24 hours, and drops of actinophage placed upon some of the streaks. The destruction of the growth of the actinomyces will prove the identity of the culture with streptomycin-producing *S. griseus*. This identification can be confirmed by the use of the agar-streak method, using various test bacteria, the sensitivity of which to streptomycin is known. Finally the cultures are grown in media used for the production of streptomycin, and the antibiotic produced is compared with streptomycin.

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16174 P

Enhancement of Heterophile and Bacterial Agglutination Titers by Means of Serum Diluent.*

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Various workers have shown that Rh agglutination titers are enhanced, and the action of the so called "blocking" antibody inhibited by the use of serum, plasma or bovine albumin instead of saline as a diluent.[1-6] Recently Griffiths[7] has reported the inhibition of Brucella blocking antibodies when rabbit serum is used in place of saline as a diluent in agglutination tests. This paper is a preliminary report on the enhancement of heterophile and various bacterial agglutinin titers by use of serum diluent instead of physiological saline. Routine agglutination tests were done comparing saline and pooled human

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5 Levine, P., *Am. J. Clin. Path.*, 1946, **16**, 597.

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or rabbit serum as a diluent. Titrations using plasma, ascitic fluid and 20% bovine albumin diluents were also carried out.

Methods. Heterophile agglutination tests were done according to the technic of Paul and Bunnell.[8] Serial twofold dilutions were made of the patient's serum in saline diluent, and equal parts of 1% sheep cells in saline were added to each tube. The tubes were shaken and incubated at 37°C for 2 hours. Next they were removed and kept overnight in the icebox at 5°C. The titer was read as the highest dilution showing distinct visible clumps of erythrocytes. The tests using human or rabbit serum diluent were carried out in an identical manner except that serum was used in place of saline. The serum diluent was inactivated by heating at 56°C for ½ hour and tested for the presence of heterophile antibodies prior to use. Only serum diluent having no heterophile agglutinins was used. The Davidsohn guinea pig kidney absorption test for infectious mononucleosis[9] was carried out in a similar manner with the patient's serum.

The bacterial agglutination tests were performed in the following manner: Serial twofold dilutions of the patient's serum previously inactivated by heating at 56°C were made both in saline and serum diluents having no agglutinins for the test organism. The serum diluent was heated at 56°C for ½ hour before use. Equal parts of various bacterial antigens suspended in saline and adjusted to a turbidity of nephelometer (McFarland) tube No. 3 were added to each tube. The tube mixtures were shaken and incubated at 56°C for 2 hours. They were then kept overnight in the icebox at 5°C and read the following morning. The following bacterial antigens were studied: typhoid, paratyphoid A and B, Shigella, Brucella and paracolon.

Results. Heterophile Agglutination. Typical heterophile agglutinin titrations comparing saline and serum diluents in individuals with positive and negative clinical and hematological† findings for infectious mononucleosis are summarized in Table I. Human plasma, ascitic fluid, rabbit serum, and 20% bovine albumin diluents also significantly enhanced heterophile agglutination titers. As shown in Table I heterophile agglutinins were detected in the serum of one patient (SK) with positive clinical and hematological findings for infectious mononucleosis using serum diluent, although repeatedly negative results were obtained in the routine saline test. In another positive case (EM) significant titers of heter-

TABLE I.
Enhancement of Heterophile Agglutination Titers by Means of Serum Diluent.

Patient	State of serum	Agglutinin titer: Saline diluent	Agglutinin titer: Serum diluent
Positive Clinical and Hematological Findings.			
EN	Un*	1:5,120	1:40,960
	Abs†	1:1,280	1:5,120
EM	Un	1:80	1:1,280
	Abs	1:20	1:320
MC	Un	1:80	1:640
	Abs	1:20	1:160
SK	Un	0	1:80
	Abs	0	1:20
LM	Un	1:320	1:2,560
	Abs	1:80	1:320
DD	Un	1:40	1:640
	Abs	1:20	1:160
SR	Un	1:160	1:1,280
	Abs	1:40	1:160
IM	Un	1:5,120	1:40,960
	Abs	1:1,280	1:5,120
FS	Un	1:80	1:640
	Abs	1:40	1:160
Negative Clinical and Hematological Findings.			
WW	Un	1:20	1:320
	Abs	0	0
WA	Un	0	0
	Abs	0	0
WM	Un	1:10	1:80
	Abs	0	0
HM	Un	1:10	1:160
	Abs	0	0
JG	Un	0	0
	Abs	0	0
CR	Un	0	0
	Abs	0	0
GN	Un	0	0
	Abs	0	0
DR	Un	0	0
	Abs	0	0
MM	Un	0	1:160
	Abs	0	0
GW	Un	0	1:320
	Abs	0	0

* Unabsorbed.
† Absorbed with guinea pig kidney suspension.

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† We are indebted to Dr. Karl Singer of the Hematology Department of Michael Reese Hospital for hematological studies.

TABLE II.
Enhancement of Bacterial Agglutination Titers by Means of Serum Diluent.

Patient	Test antigen	Agglutinin titer: Saline diluent	Agglutinin titer: Serum diluent
PN	Typhoid H	0	0
	" O	1:320	1:2,560
	Paratyphoid A	1:80	1:160
	" B	1:160	1:1,280
NL	Flexner V	1:80	1:640
	" Y	1:160	1:640
	Sonne	0	0
	Schmitz	1:80	1:640
	Shiga	0	0
JC	Flexner V	1:320	1:5,120
EG	*Brucella abortus*	1:40	1:160
	" *melitensis*	1:80	1:80
	" *suis*	1:40	1:80
JB	Typhoid O	0	0
	" H	1:320	1:1,280
	Paratyphoid A	0	0
	" B	1:160	1:1,280
CE	Paracolon	0	1:320

ophile agglutinins in both unabsorbed and absorbed serum were detected 5 days earlier with serum diluent. The confirmatory value of the guinea pig kidney absorption test in diagnosis of infectious mononucleosis[9] is emphasized by the fact that serum diluent failed to enhance titers of absorbed serums in negative cases.

Bacterial Agglutination. Preliminary results showing the enhancement of various bacterial agglutinin titers by means of serum diluent are given in Table II. It was noted that clumps of agglutinated bacteria in serum diluent were more easily dispersed on vigorous shaking than in saline. A similar enhancement of titer was observed with plasma, ascitic fluid, rabbit serum and 20% bovine albumin diluents. It is of interest that agglutinins for a paracolon bacillus isolated from a paratyphoid-like infection were demonstrable only by means of serum diluent.[10]

Comment. We feel that our results together with those previously reported by others suggest that not only does serum diluent inhibit the action of blocking antibody in certain serum, but that it is probably a more favorable medium in which the agglutination reaction can occur. Perhaps the presence of agglutinins can be detected sooner in the course of an infection by the routine use of serum diluent.

Summary. Heterophile and various bacterial agglutination titers were significantly enhanced by the use of serum diluent instead of saline. Similar results were obtained with plasma, ascitic fluid and bovine albumin diluents.

[10] Milzer, A., unpublished studies.

16175

Effect of Estrogen on Plasma Vitamin A of Normal and Thyroidectomized Rabbits.*

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The congenital malformation in the eyes of young rats produced by a severe maternal deficiency of vitamin A during pregnancy,[1,2] appears to have a strong resemblance to the fibroplasia which has been observed to occur spontaneously behind the lenses of some premature human infants.[3] Since it seems unlikely that this retrolental fibroplasia in humans is due directly to a maternal deprivation of vitamin A,[2] other influences which might cause

* This work was supported by a grant from the Foundation for Vision for the study of Retrolental Fibroplasia.

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