the motor end plates and myoplasm in response to the reflex effects of thermal trauma applied to the skin.

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15113 P

Studies on Mode of Action of Streptomycin. I. Effect of Culture Media.

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In studying the mode of action of penicillin on bacteria, Hobby, Meyer, and Chaffe[1] and Lee, Foley, and Epstein[2] observed that multiplication must take place to get killing action and that the more rapid the growth of bacteria the greater the rate of destruction. Waksman and Woodruff[3] found the reverse to be true in the case of actinomycin and streptothricin. With these two antibiotics it was more difficult to inhibit bacteria on a good medium than it was on a poorer medium. The study reported here is an attempt to determine the action of streptomycin on bacteria and to see if this action was similar to either the action of penicillin or of actinomycin and streptothricin.

The streptomycin was prepared at the University of Illinois in the laboratories of H. W. Anderson and purified and standardized in the laboratories of H. E. Carter.[4,5] The unit used is practically the same as the Waksman dilution unit.[6]

Eberthella typhosa (Hopkins strain) and *Staphylococcus aureus* (FDA 209) were used as the test organisms, and were tested against the streptomycin according to the following procedure.

The following media were compared: (1) nutrient broth, (2) nutrient broth diluted with an equal amount of water, and (3) brain heart infusion. Because the presence of phosphate and NaCl might affect the action of streptomycin it was thought not advisable to use Difco brain heart infusion, but instead the brain heart infusion was made according to the following procedure. Five hundred g of ground beef heart were infused with 1000 ml of water and 500 g of beef brain were similarly treated. These infusions were separately filtered through gauze, heated to boiling and filtered again. The medium was made from these infusions using the following formula:

Beef heart infusion	75 ml
Beef brain infusion	25 "
Peptone (Bacto)	1 g
Glucose	0.2 "

Adjusted to pH 7.0 and sterilized.

Streptomycin was added in varying amounts to tubes containing 15 ml of each of these media and *Eberthella typhosa* and *Staphylococcus aureus* were added. Twenty-four-hour broth cultures of the organism were diluted with twice their volume of sterile water in order to obtain an inoculum which would not be too heavy and 0.1 ml of this suspension was used as the inoculum. Immediately thereafter a bacterial plate count was made on each tube

1 Hobby, Gladys L., Meyer, K., and Chaffe, Eleanor, PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 277.

2 Lee, S. W., Foley, E. J., and Epstein, Jeanne, *J. Bact.*, 1944, **48**, 393.

3 Waksman, S. A., and Woodruff, H. B., *J. Bact.*, 1942, **44**, 373.

4 Carter, H. E., Clark, R. K., Jr., Dickman, S. R., Loo, Y. H., Skell, P. S., and Strong, W. A., *J. Biol. Chem.*, in press.

5 Loo, Y. H., Skell, P. S., Thornberry, H. H., Ehrlich, John, McGuire, J. M., Savage, G. M., and Sylvester, J. C., *J. Bact.*, in press.

6 Waksman, S. A., *Science*, 1945, **102**, 40.

TABLE I.

Action of Streptomycin on *Eberthella typhosa* and *Staphylococcus aureus* in Dilute Nutrient Broth, Nutrient Broth and Brain Heart Infusion.

	Viable organisms per ml in ten thousands								
	Eberthella typhosa					*Staphylococcus aureus*			
Medium	Amt streptomycin added in units	Hrs after inoculation 0	3	6	9	Amt streptomycin added in units	Hrs after inoculation 0	3	6	9
½ NB	0	20	70	*	*	0	40	40	50.	100.
,,	4.0	50	10	1.0	.5	7.5	10	6	.06	.03
,,	4.5	30	5	0.8	.3	8.0	20	7	.07	.02
,,	5.0	40	2	0.5	.3	8.5	20	7	.04	.01
NB	0	80	260	*	*	0	30	40	80.	140.
,,	4.0	40	40	4.	3.	7.5	10	4	2.	1.5
,,	4.5	40	30	6.	5.	8.0	50	4	1.	.6
,,	5.0	40	5	5.	3.	8.5	30	4	.5	.1
BHI	0	40	210	*	*	0	5	20	150.	300.
,,	4.0	60	190	*	*	7.5	15	7	3.	150.
,,	4.5	50	170	*	*	8.0	30	1.5	.7	620.
,,	5.0	40	230	*	*	8.5	20	1.3	.5	540.

NB—Nutrient Broth.
½ NB—Dilute Nutrient Broth.
BHI—Brain Heart Infusion.
* Too numerous to count.

to determine the density of the inoculum and at 3, 6, and 9 hours counts were repeated to determine the action of the streptomycin. In the first few series an attempt was made to determine the bacterial numbers nephelometrically with a Model 400 Lumetron (photoelectric colorimeter). Since the plate counts were found to be much more reliable, however, the nephelometric method was discontinued. All incubation was done at 37°C. The amounts of streptomycin found to be effective were 4.0, 4.5, and 5.0 units for *Eberthella typhosa* and 7.5, 8.0, and 8.5 units for *Staphylococcus aureus*. The streptomycin was prepared so that one unit was contained in 0.1 ml of sterile water.

Table I summarizes the results of the tests. They were repeated several times and the results checked on all occasions.

The table indicates that the action of streptomycin is not like that of penicillin but more like that of actinomycin and streptothricin as observed by Waksman and Woodruff. This was especially true with respect to cultures of *Eberthella typhosa* which showed active growth in brain heart infusion while those in nutrient broth and dilute nutrient broth rapidly decreased in numbers. There seemed to be a greater decrease in the diluted nutrient broth than in the full strength nutrient broth. The same held true for the *Staphylococcus aureus*, but the growth was slower and not as abundant in the brain heart infusion as it was in the case of *Eberthella*. In fact there was a decrease at the third and sixth hours which indicated killing action and then a marked increase. It is possible that this may mean that the streptomycin is used up in the action. This is being studied.

It is apparent that there is something present in the brain heart infusion which interferes in some way with the action of streptomycin which is not present in the nutrient broth.