0.5 to 3% were obtained. It is apparent that a level of d,l-O-heterobiotin as high as 1000 γ per 100 g of ration does not give a complete growth response in chicks on our biotin deficient ration and it appears that higher levels would not have afforded complete protection since the 1000 γ level actually gave no better response than the 500 γ level. This failure of O-heterobiotin to completely replace d-biotin for growth when it is highly active in the prevention of dermatitis indicates that the analog is capable of fulfilling only part of the function of d-biotin in the chick. On the other hand, the plateau in growth observed above the 500 γ level (compare group 11 to 10) suggests an inhibitory effect of the d,l analog on growth. In this connection, Luckey *et al.*[4] have observed an inhibitory effect of low levels of d,l-O-heterobiotin on the growth of *Streptococcus fecalis*. Further experimental data are required to clarify this point.

The analyses for d,l-O-heterobiotin indicate that this compound does not occur naturally in chicks receiving our biotin deficient diet alone or when supplemented with d-biotin. No appreciable accumulation of the d,l analog occurred in the livers of the chicks receiving O-heterobiotin until 1000 γ were included in the diet. It is interesting to note that this represents the same level at which a growth plateau was established. Similarly, no O-heterobiotin could be detected in muscle tissue until the 1000 γ level of the analog was reached.

Summary. d,l-O-Heterobiotin has biotin-like activity for the chick. Levels of 20 γ per 100 g of purified biotin-deficient basal ration give complete protection against dermatitis and some growth response. The growth activity of the oxygen analog varies inversely with the level fed and growth comparable to that on an adequate biotin diet was not obtained. d,l-O-Heterobiotin can be detected in the liver and muscle tissue of chicks fed high amounts of this compound.

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The Inactivation of Streptomycin and its Practical Applications.*†

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Because of the rapidly accumulating information on the production, purification and practical utilization of streptomycin,[1] a knowledge of the effects of experimental conditions upon its activity is increasingly important. It is known, for example, that a weakly alkaline environment, namely pH 8.0 to 8.2, is favorable to the action of streptomycin and that acidic conditions are injurious.[2] Likewise, its activity is diminished by the presence of glucose and of phosphates. Cysteine and several ketone reagents also inactivate streptomycin.[3] Since factors of this sort can be of importance in choosing media for the testing of the potency of streptomycin, in developing tests for the sterility of streptomycin preparations, and in other practical applications, it was believed desirable to reinvestigate and extend some of the earlier observations. The presence of reducing agents, such as cysteine and glucose, among the substances that inactivate streptomycin, as well as the observation that anaerobic bacteria are somewhat resistant to its activity, has suggested that special attention be paid to these conditions, in an effort to learn whether their effects are of a fundamental

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[1] Waksman, S. A., and Schatz, A., *J. Am. Pharm. Assn.*, 1945, **34**, 273.

[2] Waksman, S. A., Bugie, E., and Schatz, A., *Proc. Staff Meet. Mayo Clinic*, 1944, **19**, 537.

[3] Denkewalter, R., Cook, M., and Tishler, M., *Science*, 1945, **101**, 12.

TABLE I.
Effect of Atmosphere Upon Action of Streptomycin.

Atmosphere	Activity in dilution units per ml against			
	E. coli	*A. aerogenes*	*Pr. vulgaris*	*S. marcescens*
Air	500	500	500	500
H_2	200	400	100	20
N_2	100	100	100	80
CO_2	20	20	30	20

TABLE II.
Effect of Bicarbonate and Acidity Upon Action of Streptomycin.

	Activity in units/ml against			
	E. coli	*A. aerogenes*	*S. aureus*	*B. subtilis*
Nutrient agar (pH 6.8)	100	100	100	1,000
Nutrient agar (pH 5.7)	10	10	5	100
Nutrient agar + 2% $NaHCO_3$	100	100	100	800

nature or are due to secondary factors such as fall in pH value.

Streptomycin is a rather stable compound. No biological agent or enzyme system has yet been found capable of destroying it. At 25°C, it is not affected appreciably between pH 2.0 and 9.0, but it is decomposed by excessive acidity (1 *N* HCl) and alkalinity (0.1 *N* NaOH). Hydrolysis by hydrochloric acid in methanol has been shown to result in the splitting of the molecule with the liberation of a base, streptidine, and a carbohydrate-like substance, streptobiosamine, neither of which possesses much, if any, antibiotic activity. The streptidine is believed to be attached to the streptobiosamine by a glucosidic linkage.[4]

Aerobic vs. Anaerobic Environment. In order to learn whether the greater resistance of anaerobes than of aerobes to streptomycin was due to the nature of the organisms or to the environment, a study was first made of the activity of streptomycin on several bacteria growing in different environments. Organisms that are facultative anaerobes, *i.e.*, that can grow either in air or with a limited supply of oxygen, were selected. A highly active streptomycin preparation containing 500 units/ml was used. Serial dilutions of the streptomycin solution were made in nutrient agar, and 4 series of plates were made up with this streptomycin-containing agar and streaked with the 4 test bacteria. One series of plates was incubated at 28°C under the usual aerobic conditions, and the other 3 series were incubated under anaerobic conditions in atmospheres of hydrogen, nitrogen, and carbon dioxide. The results presented in Table I show that streptomycin is about one-half as active under hydrogen as in air, considerably less active under nitrogen, and almost completely inactive under carbon dioxide.

Two supplementary tests were made in an effort to elucidate the effect of carbon dioxide. First, it was determined that the pH of nutrient agar was changed from 6.8 to 5.7 by storage for 16 hours under carbon dioxide. Tests showed that on agar adjusted to this pH, streptomycin had its activity diminished to about the same degree as by carbon dioxide. The effect of streptomycin was now tested on nutrient agar to which sodium bicarbonate had been added (Table II). No inactivation of streptomycin took place. One may, therefore, conclude that the effect of carbon dioxide is probably a result of the change in pH produced by it in the medium.

In order to determine whether the di-

[4] Brink, N. G., Kuehl, Jr., F. A., and Folkers, K., *Science*, 1945, **102**, 506.

TABLE III.
Changes in pH Value Produced by 4 Test Bacteria in Different Environments.

Atmosphere	Organism	pH*
Air	*E. coli*	7.50
	A. aerogenes	7.80
	Ser. marcescens	7.35
	Pr. vulgaris	7.13
Nitrogen	*E. coli*	7.25
	A. aerogenes	7.54
	Ser. marcescens	6.68
	Pr. vulgaris	6.69
Hydrogen	*E. coli*	6.63
	A. aerogenes	6.84
	Ser. marcescens	6.48
	Pr. vulgaris	6.43

* Original pH 7.0.

TABLE IV.
Effect of Sugars Upon Susceptibility of *B. subtilis* to Streptomycin.

Sugar added*	pH after growth	Cup method zone size in mm
None	7.1	19.3
Dextrose	6.3	10.1
Levulose	6.3	10.0
Sucrose	5.3	10.4
Mannose	7.1	18.4
Galactose	7.1	17.8
Arabinose	6.9	14.2
Maltose	7.0	18.5
Lactose	7.1	17.2
Mannitol	6.7	10.0

* 1% in nutrient agar.

minished activity of streptomycin in nitrogen or hydrogen could be a result of the production of acid by the test organisms under these conditions, a study was made of the changes in pH of the cultures. Flasks of nutrient broth were inoculated with the above 4 cultures and kept under air, hydrogen, and nitrogen. After incubation for 24 hours, pH determinations were made (Table III). The results suggest that the production of acid by the test bacteria under anaerobic conditions may be at least one factor in the diminished activity of streptomycin under these conditions.

Effect of Sugars. The action of streptomycin on *B. subtilis* is known to be diminished by glucose.[1] In order to obtain further information concerning this effect, the following 2 experiments were conducted. Since glucose is fermented by *B. subtilis* with the formation of acid, and since streptomycin is less active at low pH, it seemed likely that the action of glucose was largely a pH effect. A study was therefore made of the production of acid by *B. subtilis* from several sugars, as well as the effect of these sugars upon the activity of streptomycin. The sugars were incorporated in nutrient agar, which was inoculated with a spore suspension of *B. subtilis,* and plates were poured. Metal cups were placed upon the solidified agar plates and the streptomycin solution was added. The results presented in Table IV show that those sugars, namely dextrose, levulose, and sucrose which yield acid diminish most markedly the action of streptomycin. Mannose, galactose and lactose, from which little or no acid is produced, have little effect upon the activity of streptomycin.

When *E. coli* was used as the test organism (Table V) it was found that acid was produced by this particular strain from all the sugars, but in the smallest amount from lactose. Likewise all the sugars but lactose gave a diminution in the effectiveness of streptomycin against this organism.

That the inactivating effect of glucose upon streptomycin is due to the production of acid has been further confirmed by the following experiment: when the pH of the test medium was held at 8.0 by the addition of a glycine buffer, the addition of glucose did not result in the inactivation of streptomycin. Glycine alone had no effect upon the activity of streptomycin.

In view of observed effects of cysteine, the

TABLE V
Effect of Sugars Upon Susceptibility of *E. coli* to streptomycin.

Sugar added*	pH after growth	Cup method zone size in mm
None	6.5	17.5
Dextrose	5.1	15.0
Levulose	5.1	16.0
Sucrose	5.0	15.5
Mannose	5.0	15.0
Galactose	5.2	15.2
Arabinose	5.2	17.0
Maltose	5.3	16.8
Lactose	6.1	18.0
Mannitol	5.2	16.5

* 1% in streptomycin agar.

TABLE VI.
Effect of Cevitamic Acid Upon Action of Streptomycin.

Cevitamic acid	Activity, in dilution units per gram, against			
	E. coli	*A. aerogenes*	*S. aureus*	*B. subtilis*
0	100	100	50	800
0.4 mg/ml	10	10	5	100

TABLE VII.
Effect of Sulfhydryl Compounds on Action of Streptomycin.

Sulfhydryl compound	Zone size, mm	
	*B. subtilis**	*E. coli*†
None	18.0	27.0
Cysteine (0.02 *M*)	10.0	8.0
Thioglycolate (0.04 *M*)	17.0	25.5
Monothioglycol (0.04 *M*)	—	22.0
Glutathione	18.0	—

* Streptomycin concn. 25 units/ml medium, nutrient agar.
† Medium, streptomycin agar.

effect of another reducing agent—cevitamic acid—upon the activity of streptomycin was studied. The tests were made by the agar-streak method, using nutrient agar at pH 6.8 and nutrient agar containing 0.4 mg of cevitamic acid per ml and adjusted to pH 6.8. The results (Table VI) show that cevitamic acid greatly diminishes the effectiveness of streptomycin. Tests for the production of acid from cevitamic acid of this concentration showed that no detectible acid was produced by the test organisms. The cause of inhibition by the cevitamic acid is thus not immediately apparent.

Effect of Sulfhydryl Compounds. According to Denkewalter, Cook and Tishler[3] cysteine and β-mercaptoethylamine inhibited the action of streptomycin on *B. subtilis,* whereas thioglycolic acid was ineffective. A detailed study was made of this phenomenon, which fully confirmed their results and permitted the extension of their conclusions to include also the gram-negative *E. coli* (Table VII). It was also found that cysteine does not stimulate the production of acid by these organisms.

Thioglycolate agar (Brewer's) has been proposed as a medium for use in testing streptomycin for sterility. This medium contains not only thioglycolate, but also glucose, and is far richer in nitrogenous constituents than the usual nutrient agar. Accordingly, the effects of these constituents upon the activity of streptomycin were studied. The results of several experiments (Table VIII) show that the presence of 0.2% of sodium thioglycolate in nutrient agar did not inactivate streptomycin. Whereas the presence of 1.0% of glucose diminished the bacteriostatic effect of streptomycin. Glucose plus thioglycolate had no greater effect than glucose alone. With an increase in the concentration of the nitrogenous constituents in the medium, streptomycin was found generally less effective, but the absence of inactivation by thioglycolate, diminution of activity by glucose, and absence of synergistic action of the 2 substances were again observed.

Effect of Ketone Reagents. Brink, Kuehl and Folkers[3] have reported that "when streptomycin was treated with a variety of carbonyl group reagents, complete inactivation was observed." They mentioned particularly hydroxylamine and semicarbazide, and have isolated the oxime and semicarbazone. To confirm their results, solutions of streptomycin were mixed with the ketone reagents and the mixture was tested by the cup method (Table IX). Certain amino compounds which are not ketone reagents, such as ethylenediamine and methylamine, were without effect upon the activity of streptomycin.

Age of Culture. Streptomycin appears to have a more marked effect upon young actively growing cultures of bacteria than upon older cultures where the rate of growth has diminished. To confirm this, streptomycin was added to cultures of *E. coli* in nutrient broth which contained streptomycin, and after 8 hours' further incubation at 28°C, the number of viable cells were counted.

It was found that when streptomycin was

TABLE VIII.
Effect of Thioglycolate and Glucose Upon Action of Streptomycin.

Nutrient agar—agar-streak method

Substance added	Dilution units against *E. coli*	*S. aureus*	*B. subtilis*
None	100	50	1000
Glucose*	30	20	200
Thioglycolate	100	50	1000
Glucose+ thioglycolate	30	20	30

Nutrient agar—cup method

	Zone of inhibition, mm *B. subtilis*	*E. coli*
None	16.5	16.7
Glucose	10.0	14.5
Thioglycolate	15.3	16.2
Glucose+ thioglycolate	10.0	15.7

Enriched agar†—agar-streak method

	Dilution units against *E. coli*	*S. aureus*	*B. subtilis*
None	10	50	300
Glucose	5	10	10
Thioglycolate	20	20	200
Glucose+ thioglycolate	10	10	50

* Glucose added in 1% concentration, and thioglycolate in 0.2%.
† Composition of enriched agar:

Beef extract	5 g
Peptone	20 g
NaCl	5 g
Agar	20 g
Dist. water	1000 ml

added to cultures from 0 to 3 hours old, a 75% reduction in growth resulted when the concentration of streptomycin was 2 units per ml and a 95% reduction in growth when the concentration was 5 units per ml. In cultures 24 to 48 hours old, the effect of streptomycin was less marked. A concentration of 2 units per ml caused a reduction in growth of 19%, and a concentration of 5 units per ml a reduction of 42%.

TABLE IX.
Effect of Ketone Reagents Upon Action of Streptomycin (25 units/ml).

Ketone reagent	Zone—size, ml *B. subtilis*	*E. coli*
None	18	25
Hydroxylamine (.002 *M*)	10.5	8.0
Hydrazine (.002 *M*)	—	10.0
Semicarbazide (.002 *M*)	9.0	—
Phenylhydrazine (.002 *M*)	13.0	8.0
Methylphenylhydrazine (.001 *M*)	15.0	10.0

Discussion. The antibacterial activity of streptomycin can be largely or completely neutralized or antagonized by various chemical agents. These include glucose and certain other sugars, an anaerobic environment, certain sulfhydryl compounds, and ketone reagents. In some cases, as in the action of sugars or the anaerobic environment, the effect on streptomycin can be traced to the acidity produced under these particular conditions. However, in the effect of cysteine, cevitamic acid, and of ketone reagents the

inhibition of streptomycin activity may be associated with the blocking of an active grouping in the molecule of the streptomycin. To explain the inactivation of the antibacterial properties of streptomycin by the blocking of a single group in its molecule cannot be attempted at present due to its rather complicated structure. Until the chemistry of streptomycin is more clearly elucidated, it is difficult to present a suitable theory that would account for the various effects of streptomycin inactivation.

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Blood Platelets in Heterophil Anaphylaxis.

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The serum of rabbits into which erythrocytes of sheep have been injected produces toxic symptoms and death when injected into guinea pigs. The reason for this was explained when Forssman[1] and others[2,3] announced the discovery and distribution of the so-called heterophil antigen. At this time it was found that the same antigenic substance which is present in erythrocytes of sheep is also present in the tissues of the guinea pig, dog and other species. Hence, the antibody produced in the rabbit in response to erythrocytes of sheep would also react with the antigen present in the tissues of the animal injected.

As the reactions exhibited by the animals following such injections of serum are almost identical with those of anaphylaxis, the phenomenon was designated heterophil anaphylaxis.

Later Taniguchi[4,5,6] observed that the lungs of guinea pigs killed by such injections of serum were markedly hemorrhagic and both lungs and trachea were filled with frothy fluid but were not markedly emphysematous as in true anaphylaxis. Furthermore Redfern[7] observed that no contraction is produced in the perfused uterine muscle following the addition of anti-Forssman serum. I have confirmed these facts shown by previous investigators. Moreover I found in collaboration with Recarte[8] that no increase of blood histamine was present in the acute shock induced in guinea pigs by the injection of anti-Forssman serum and no histamine was liberated when lungs of guinea pigs were perfused with such serums.

The experiments reported in the present paper were made with the aim of studying the modifications of the blood platelets in this particular type of shock. For this the volume of blood platelets was determined in guinea pigs and dogs before and after the injection of rabbit serum with high titer of antisheep hemolysin. At the same time the influence of injections of hepatic glycogen on the course of this type of shock was observed, since this substance in certain doses produces a temporary disappearance of the platelets of the blood.[9]

Methods. Thirteen unanesthetized male guinea pigs weighing from 500 to 1,030 grams were injected in the vein of the penis with vari-

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