bacterium tuberculosis, was comparable to that of streptomycin.

Isolation of the active substance has been obtained by methods used to isolate streptomycin. The methyl alcohol formic acid procedure yielded preparations assaying 158 streptomycin units per mg.

The crude preparation thus isolated from the culture filtrate of *S. bikiniensis* had no activity against fungi. Bacteria made resistant to streptomycin were also resistant to this substance. It was inactivated by cysteine, it gave reduced activity in the presence of glucose, and it was sensitive to increased acidity to the same degree as streptomycin. These results tend to establish the fact that the new antibiotic is similar to streptomycin if not identical with it.

The toxicity of the preparation was determined by the use of chick embryos. As much as 600 units of the material, deposited upon the chorio-allantoic membrane, and concentrations greater than 1200 units injected into the yolk sac, of 11-day embryos gave 100% survival. One may thus conclude that the minimum lethal dose of the new antibiotic is relatively high, at least in the chick embryo.

As long as this preparation has not been crystallized and as long as its activity *in vivo* against various bacteria has not been determined, it is not possible to state definitely that the new antibiotic is streptomycin. Some question may also be raised concerning its absolute identity with streptomycin, since it is produced by a totally different organism and occasional quantitative differences in the degree of sensitivity of certain bacteria to this antibiotic as compared with streptomycin are obtained. For these reasons, it is proposed to designate the new preparation as Streptomycin II.

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Preparation of Hemoglobin Solutions Containing Hemoglobin Reducing Enzymes.

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Employing the following procedure we are able consistently to obtain sterile solutions, free from cellular debris, containing enzymes which bring about the consumption of the entire oxygen content of the solution, thereby converting the hemoglobin present into the extremely stable reduced form.

Procedure. Human erythrocytes are separated from the plasma by means of a bucket centrifuge. These cells are suspended in 2 volumes of a solution containing 6% glucose, 0.15% nicotinic acid amide and 0.0006% ammonia. This suspension is centrifuged at approximately 20,000 r.p.m. in a Sharples separator-type bowl that separates the cells from the wash solution and at the same time lyses them, though with little loss of hemoglobin into the wash solution. The washed, lysed cells, having a hemoglobin concentration of approximately 30%, are delivered directly into 100 cc of distilled water containing sufficient nicotinic acid amide to produce a concentration of 0.15% in the final product, which is diluted to contain approximately 7% hemoglobin. The mixture is diluted with 2 volumes of distilled water containing sufficient glucose to produce an estimated final glucose concentration of 6%. The diluted mixture is adjusted to pH 5.8 with N/10 HCl and is centrifuged through a Sharples clarifier-type bowl to remove the stromata. The centrifugate is stirred for 30 minutes with "Decalso," 30 g per liter, from which it is decanted. An amount of normal NaOH solution calculated to neutralize the HCl is added. These last 3 steps have been adopted from the work of Hamilton and Farr.[1] Am-

[1] Hamilton, P. B., and Farr, L. E., *Fed. Proc.*, 1946, **5**, 136.

TABLE I.

Sol'n No.	Total Hb, %	% of total Hb. appearing as MetHb. after storage at room temp. for period indicated								% oxyhemoglobin of the solutions stored at 37°C for period indicated				
		Initial	2 days	3 days	6 days	7 days	15 days	16 days	39 days 40 days	Initial	1 day	2 days	3 days	4 days 11 days
1029-D3	7.36	0.52	1.3			1.89	0.55		1.5	7.79	6.57	4.55		1.59 0
1029-D6	6.36	3.08		15.62	18.3			38	73.2	6.12	1.59		0.46	0

monium hydroxide is added in the amount of 0.6 mg of NH_3 per 100 cc. Clarifying and sterilizing filtrations are carried out using Republic K6 and S6 pads. The entire procedure is carried out at 0°C in the course of 8 hours.

Solution 1029-D3 is an example of solutions produced as outlined above. Its behavior during storage was compared (Table I) with that of a solution in the preparation of which the use of ammonia was omitted (Solution 1029-D6). Other modifications of the procedure (elimination of nicotinic acid amide, elimination of glucose, use of isotonic saline, etc.) gave results closely resembling those of 1029-D6.

The Evelyn photoelectric colorimeter was used for the measurement of total hemoglobin and methemoglobin, as described by Evelyn and Malloy.[2] The oxygen content of the solutions was measured by the technic of Van Slyke and Neill.[3]

At the end of 15 days at room temperature, or 7 days at 37°C, Solution 1029-D3 had assumed the "grape-juice" color of reduced hemoglobin, which color was retained for months. During the storage experiments, 1029-D6 became progressively browner, until after 39 days at room temperature and 4 days at 37°C the brown methemoglobin almost completely masked the brilliant red of the remaining oxyhemoglobin.

Summary. Hemoglobin solutions were prepared from erythrocytes that were laked in the presence of nicotinic acid amide, glucose, and ammonia. The solutions were Seitz-filtered, yielding clear solutions free from cellular debris, and containing sufficient of the enzyme systems of the cell to change the pigment from oxyhemoglobin to reduced hemoglobin with no appreciable accumulation of methemoglobin during the process. The solutions could be stored in the reduced state for protracted periods at elevated temperatures without increase in methemoglobin content.

[2] Evelyn, K. A., and Malloy, H. T., *J. Biol. Chem.*, 1938, **126**, 655.

[3] Van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 1924, **61**, 523.