This degradation has been carried out using nonradioactive glucose, but no difficulties are anticipated in applying it to the radioactive compound. The nitration is necessary because compound VIII without the nitro group is an oil, not as easily isolated or characterized as the crystalline derivative. In using radioactive glucose a barium hydroxide trap should be attached to the dry distillation of compound VII, the final step in degradation. Silver carbonate, formed in this reaction, decomposes at 218° C with liberation of carbon dioxide. The trapping and counting of this gas would confirm the radioactivity on C-3 of the glucose. Heating aqueous solutions in recrystallizations should be done under reflux because some osotriazole derivatives are volatile in steam. All compounds formed in this degradation are well characterized crystalline compounds, soluble in organic solvents such as chloroform, ethanol, pyridine and acetone.

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The Adaptation of the Voges-Proskauer Reaction for the Quantitative Assay of Streptomycin

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At the present time there are several chemical methods for the assay of streptomycin. The principal chemical methods are the tests of Sullivan and Hilmer (*1*), Boxer, Jelinek, and Leghorn (*2*), and Marshall *et al.* (*3*). The test of Sullivan and Hilmer (*1*), is not sensitive with concentrations of the antibiotic lower than 1000 units/ml and values are high due to breakdown products formed during the test. The methods of Boxer, Jelinek, and Leghorn (*2*), and Marshall (*3*) are excellent chemical assay methods for streptomycin, but neither is adaptable to the assay of dihydrostreptomycin. All 3 of these assay methods require considerable time to perform.

The Voges-Proskauer reaction depends on the production of acetylmethylcarbinol or acetoin from glucose metabolism. In the presence of potassium hydroxide, acetylmethylcarbinol is oxidized to diacetyl which reacts with substances containing a guanidine residue to give a red-colored compound. Streptomycin and dihydrostreptomycin both contain 2 free guanido groups, which might be expected to complete this reaction. The purpose of this paper is to report a modification of the Voges-Proskauer reaction that permits a rapid quantitative assay for either form of the antibiotic.

Modifications of the Voges-Proskauer reaction such as the methods of Harden and Norris (*4*), Barritt (*5*), O'Meara (*6*), and Coblentz (*7*) were investigated to determine their desirability for use in the assay of either form of the antibiotic. Acetylmethylcarbinol (Matheson Co.) was used for preliminary work but, because of its unstable nature, diacetyl (Eastman Distillation Products Industries) was later adopted as the reagent of choice. These various modifications of the Voges-Proskauer reaction were repeated many times, using varying amounts of reagents, different concentrations of reagents, and different sequences of addition of reagents. After optimum conditions had been determined, tests were carried out with different concentrations of streptomycin and dihydrostreptomycin to determine the sensitivity of the assay. Color readings were made with a Klett-Summerson photoelectric colorimeter using filters #42, 54, and 66.

Except for the modifications of Harden and Norris (*4*) and Barritt (*5*), all the procedures of the Voges-Proskauer reaction proved to be unsatisfactory because of lack of sensitivity. After modification of these 2 methods the following procedure was developed. The amounts and concentrations of reagents were placed in Klett-Summerson tubes in the following order.

Streptomycin (varying concentrations)	1.0 ml
Alpha naphthol (5% in 95% ethanol)	0.5 ml
Potassium hydroxide (40%)	0.1 ml
Distilled water	2.9 ml
Diacetyl (1-1000 dilution)	0.5 ml

The function of potassium hydroxide in the Voges-Proskauer reaction is thought to be that of oxidation of acetylmethylcarbinol to diacetyl. Therefore, it would seem that if diacetyl were used, potassium hydroxide would not be needed. It was found that no color developed if potassium hydroxide was omitted. This indicates a new and unexplained function of potassium hydroxide in the reaction. It was found that the addition of streptomycin first and diacetyl last will give positive results whereas any other order of addition will give negative results. The tubes were shaken for 10 min to develop maximum color and then read for transmittency percentage in the colorimeter, using the #54 green filter. Maximum color was found to be stable for 15 min and tubes must be read within this time. In order to determine concentrations of streptomycin or dihydrostreptomycin greater than 1000 units/ml, 1.0-ml samples must be diluted to a total volume of 20.0 ml with distilled water, and sample amounts may then be assayed. Concentrations as low as 25 units/ml may be determined for either form of the antibiotic. This procedure gives a reproducible quantitative color test which fulfills Beer's law for any concentration from 25 units/ml to 1000 units/ml.

A standard curve can be developed by which unknown samples of the antibiotic may be assayed.

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Liver Function and Bromsulfalein Disappearance[1]

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In the bromsulfalein test of liver function, a known amount of the dye (which is designated "BSP") is injected intravenously, and its subsequent concentration in the blood is measured after one or more selected time intervals. MacDonald published both normal and abnormal curves of BSP disappearance in humans (*1*). Before 1947 it was assumed that BSP disappearance from the blood is caused only by its removal in the liver and excretion in bile. However, in 1947, Cohn, Levine, and Streicher published the results of experiments with dogs that provide good evidence of extrahepatic uptake of BSP (*2*). Those results support the following approximate interpretation of the BSP disappearance curves.

Let $y = y(t)$ and $z = z(t)$ be the amounts at time t (in min) of BSP in the blood and in the extrahepatic, extravascular tissues, respectively. Assume that all rates of BSP transfer are proportional to the amount of BSP from which the transfer occurs. Let k_1, k_2, and k_3 be the proportionality constants for the transfers from y to z, from z back to y, and from y to the liver excretion, respectively. Then

$$\begin{aligned} \frac{dy}{dt} &= k_2 z - (k_1 + k_3) y, \\ \frac{dz}{dt} &= k_1 y - k_2 z, \\ z(0) &= 0, \text{ and } y(0) = y_0. \end{aligned} \tag{1}$$

This differential system has a solution of the form

$$y(t) = Ae^{-r_1 t} + Be^{-r_2 t}, \tag{2}$$

where $-r_1$ and $-r_2$ are roots of the algebraic equation in r,

$$r^2 + (k_1 + k_2 + k_3) r + k_2 k_3 = 0,$$

and

$$A = (r_1 - k_2) y_0 / (r_1 - r_2) \text{ and } B = (k_2 - r_2) y_0 / (r_1 - r_2).$$

Also,

$$r_1 + r_2 = k_1 + k_2 + k_3 \text{ and } r_1 r_2 = k_2 k_3,$$

so that

$$k_1 + k_3 = (Ar_1 + Br_2)/y_0,$$

$$k_2 = (Br_1 + Ar_2)/y_0, \tag{3}$$

$$k_3 = r_1 r_2 / k_2 = r_1 r_2 y_0 / (Br_1 + Ar_2), \text{ and} \tag{4}$$

$$k_1 = (k_1 + k_3) - k_3 = (Ar_1 + Br_2)/y_0 - r_1 r_2 y_0 / (Br_1 + Ar_2). \tag{5}$$

In applying these results to an experimental curve, it is first determined what values of A, B, r_1 and r_2 cause equation (2) to fit the experimental results. When the observed points are plotted on semilog paper the later portion, say for $t > 30$, is approximately linear and a measure of the term $Be^{-r_2 t}$—so this segment gives r_2 from its slope and B as its extrapolation at $t = 0$. (For r_2, if $y(t_1)$ and $y(t_2)$ are on that segment and such that $2y(t_2) = y(t_1)$ then $r_2 = 0.693/(t_2 - t_1)$. If the blood volumes is V in cc, if the dose is D in mg, and if y is measured in mg/100 cc of blood, then

$$A = y_0 - B = 100\, D/V - B.$$

In some cirrhotics, for example, the blood volume is not given accurately by the usual tables relating blood volume to weight and height. In these cases A is determined more accurately from the quantity $(y_0 - B)$ with y_0, being the extrapolation of the $y(t)$ curve back to about $t = 2$, which allows a couple of minutes for the initial mixing after injection. (This extrapolation is best done on the semilog plot.) The value of r_1 is determined in the same way as r_2, but from the best straight-line fit of the semilog plot versus time of the quantity (the observed value—the value of the above $Be^{-r_2 t}$ at the time of the observation). With these observed constants one calculates the k's by using equations (3), (4), and (5).

The initial slope on the semilog plot of the observed curve is a rough measure of k_3—especially if the dose is small and if the tested individual is normal in having a k_3 that is large relative to k_1 and k_2. According to this measure and MacDonald's results (*1*) the k_3 values of normal individuals are between 0.14 and 0.4 when the dose is 2 mg/kg and between 0.075 and 0.25 when the dose is 5 mg/kg. The k_3 values appear to be less in individuals with diseased or impaired livers —k_3 may even be as low as about 0.01 (Fig. 7, curve 7 of ref. 1).

In routine testing for impaired liver function it would be best to take measurements every 5 or 10 min for about half an hour and every 10 or 20 min for at least another half hour. This is recommended because in cases with impaired liver function the values of k_1 and k_2 may be greater than normal (possibly

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