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

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Manuscript received November 17, 1952.

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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[2] Sponsored by the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions of the author do not necessarily reflect the opinion and policy of the Veterans Administration.

[3] The author thanks Donald S. Amatuzio for helpful discussions and for preliminary communication of experimental results being obtained by him and his associates at the Veterans Administration Hospital.

due to some compensatory mechanism) and thus necessitate the determination of all the constants in equations (2) and (4). Such an analysis would require a minimum of 4 determinations, at about 3, 10 or 15, 30 or 40, and 60 or 80 min. In other words, the analysis of BSP curves for abnormals (mild or severe) is much more complicated than that of normal BSP curves if the results are to be equally accurate and clearly definitive. In case the above steps of the curve analysis are omitted or improperly done, the more likely error is the underestimation of the extent of liver impairment.

The present interpretation gives an approximate confirmation of Goodman's conclusions about normal cases, but his results for abnormal cases would sometimes be too high (*3*).

The present theory also applies to the hepatectomized dogs of Cohn, Levine, and Streicher (*1*). In that case $k_3 = r_2 = 0$ and the last term of equation (2) is constant.

This treatment neglects the reabsorption factor described by Lorber and Shay (*4*). That factor should give rise to theoretically low values of k_3 (and higher values of k_1 and k_2), but these would still be correct in regard to over-all physiological significance.

If E is the efficiency with which the liver removes BSP from the blood flowing through it, and if F is the fraction of the total blood volume that flows through the liver each minute, then $k_3 = EF$. Since $0 < E < 1$ it follows that k_3 is an upper limit for liver blood flow, F. However, k_3 itself is the physiologically significant quantity, or measure, of this particular liver function.

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Manuscript received December 3, 1952.

The Influence of Iodoacetate on the Sodium and Potassium Content of *Ulva lactuca* and the Prevention of Its Influence by Light[1]

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The influence of glycolytic inhibitors on cation equilibria and movements in living cells has been investigated by Wildbrandt (*1*), Harris (*2*), and Maizels (*3*) on human erythrocytes, Dean (*4*) on muscle, Dixon (*5*) on brain cortex, and Scott *et al.* (*6*) on baker's yeast. An interpretation of the action of these agents has been based on their inhibitory effect on specific enzyme systems of carbohydrate metabolism which are associated with ion transport and equilibria.

The experiments to be reported represent a study of the influence of one of the glycolytic inhibitors, monoiodoacetate, on the sodium and potassium content of the green alga, *Ulva lactuca*. The study was undertaken to test the applicability of the postulated role of glycolysis in cation regulation in this form.

The cells of this marine organism, like most cells living in a high Na^+ low K^+ medium, accumulate K^+ and partially exclude Na^+. Since the alga consists of large membranous fronds of two layers of cells, it is particularly well suited for investigations involving ion interchange between the cell and its environment.

The *Ulva,* collected from the Eel Pond in Woods Hole, was conditioned before use under incandescent illumination in running sea water. Small uniform samples cut from a single frond were placed in large finger bowls of sea water, containing the inhibitor when present, and maintained in the dark or in the light at the temperature of running sea water (*ca.* 21° C). Samples were removed at various time intervals, rinsed for 1 min in isotonic sucrose (0.6 *M*) to remove adhering salts. The sucrose solution was then removed from the surface by a consistent blotting procedure. A wet weight was determined on the blotted material and, after drying for 12 hr at 110° C, a dry weight was taken. Cell water was calculated by difference. The dried material was ground in a mortar and extracted in 50.0 ml of 10% trichloracetic acid for a few hours. The observation has been made in our laboratory that Na^+ and K^+ are quantitatively extracted from the material by this method as compared with the usual wet ashing techniques. The extracts were analyzed for Na^+ and K^+ by flame photometry, using the Beckman spectrophotometer.

Influence of sodium iodoacetate on cellular potassium in the dark and in the light. The presence of the inhibitor in a concentration of 0.001 *M* results in a marked loss of K^+ from the cells over a period of 24 hr in the dark. Control samples taken at the beginning and end of this period were essentially constant in potassium content (Fig. 1). In the presence of light from a 100-w incandescent lamp placed at a distance of about 1 ft from the alga, the inhibitor is completely ineffective in causing loss of K^+. Rather, the potassium content of the experimentals temporarily increases over that of the controls.

To evaluate further the influence of light on the prevention of the iodoacetate effect, the concentration of the inhibitor was raised to 0.005 *M*. Again the light prevented the loss of K^+ (Fig. 1).

Influence of sodium iodoacetate on cellular sodium in the dark and in the light. Concomitant with the K^+ loss caused by the 0.001 *M* NaIAA in the dark the

[1] This paper represents part of the research performed under Contract AT(11-1)-181 between the Atomic Energy Commission and Oberlin College.

[2] The authors wish to acknowledge the technical assistance of William De Witt Andrus.