weight 50.5 kg, height 165 cm, body surface 1.54 m². The estimated hourly dose was 10.0 ml, which in 52 hr resulted in a blood alcohol of 199 mg %. This would require an excess of 93 ml of alcohol; this, subtracted from the amount administered, leaves 427 ml metabolized in 52 hr, or 197 ml daily. Decreasing the dose to 8.0 ml hourly resulted in a drop of blood alcohol of 51 mg % in 59 hr, indicating an excess of metabolism over intake of 24 ml, which, added to the dose administered, gives a total of 496 ml in 59 hr, or 202 ml daily.

Subject 4 was a chronic alcoholic, male, age 39, weight 68.2 kg, height 186 cm, body surface 1.92 m². The estimated hourly dose of 12.5 ml did not cause measurable amounts of alcohol to appear in the blood in 28 hr, so it was raised to 17.5 ml, which in 48 hr raised the blood alcohol to 63 mg %. The amount of alcohol required to produce this rise, 39 ml, subtracted from the amount administered, gives 801 ml metabolized in 48 hr, or 400 ml daily. Further increase of the dose to 20.0 ml hourly raised the blood alcohol an additional 123 mg % in 21 hr, requiring an excess of 79 ml. The amount administered, 420 ml, less this excess, gives 341 ml metabolized in 21 hr, or 389 ml daily.

From the foregoing it can be seen that there is a wide range of maximal daily metabolism in different individuals, varying in our small series from about 200 to 400 ml 95% alcohol. When this is calculated on a basis of body weight the spread is much less, but this is not further reduced when related to body surface, as can be seen from Table 1. Neither can it be

TABLE 1

Subject	1	2	3	4
Sex	Male	Male	Female	Male
Alcohol habits	Moderate	Moderate	Excessive	Excessive
Daily metabolism 100-proof liquor (ml)	608	524	383	760
Daily metabolism g alcohol/kg	4.17	3.23	3.06	4.46
Daily metabolism g/m²	151	117	100	158

related to drinking habits, since the values for the two moderate drinkers lie between the extremes shown by the chronic alcoholics.

It seems unlikely that maintaining the average blood alcohol level in a higher range would be effective in increasing to an appreciable degree the maximal metabolism of alcohol. Thus in the case of subject 3, during the first 52 hr the average blood alcohol concentration was about 100 mg % and the amount metabolized daily, 197 ml, whereas during the second period of 59 hr the average blood alcohol was 175 mg % with an almost identical daily consumption of 202 ml. This is in accord with the findings of Cutting, Newman, and Yee (*4*) that there is some increase in rate of alcohol metabolism with increasing blood alcohol concentration, but that this is minor in degree.

In all the foregoing the term metabolism has been used to cover the disappearance of all the ingested alcohol. This is obviously inaccurate, since some is excreted, mainly in the breath and urine, but since this is at most 10% it can for practical purposes be disregarded.

We can thus conclude that administration of alcohol over a period of a number of days has demonstrated a maximum metabolism in our series equivalent to 760 ml of 100-proof liquor, somewhat less than the quart estimated from animal work. No doubt a larger series would show a greater range, but it is unlikely that the quart a day would be significantly exceeded.

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On the Evaluation of the Constants V_m and K_M in Enzyme Reactions

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The Michaelis-Menten theory (*1*) of enzyme action may be expressed by the following equation:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} C \xrightarrow{k_3} E + P, \qquad \text{(I)}$$

where E is free enzyme, S is free substrate, C an intermediate enzyme-substrate complex, and P the product of the reaction. Using the same symbols for denoting concentrations, it follows that the reaction rate (v) is at any moment equal to $k_3 \cdot C$. When $S \rightarrow \infty$, the reaction rate approaches a maximum (V_m) equal to $k_3 \cdot E_t$, where $E_t = E + C$. If initial reaction rates are measured, the substrate concentration will remain practically constant during the time of measurement. Further, if $S \gg E_t$, only a negligible amount of substrate can accumulate in the form of the intermediate complex. From this it follows that (a) the concentration of free substrate may be set equal to the total substrate concentration, and (b) the rate of disappearance of substrate equals the rate of formation of product (steady state). Therefore, $k_1 \cdot E \cdot S - k_2 \cdot C = k_3 \cdot C$. By substitution and rearrangement one obtains

$$\frac{(V_m - v)S}{v} = \frac{k_2 + k_3}{k_1} = K_M \text{ (Michaelis constant).} \qquad \text{(II)}$$

Numerically, K_M is equal to the substrate concentration at half-maximum reaction rate ($V_m = 2v$). When $k_2 \gg k_3$, K_M may be set equal to the dissociation constant (k_2/k_1) of the enzyme-substrate complex, and $\frac{1}{K_M}$ then becomes the "affinity" constant.

Equation II can be written in a linear form in the following three ways:

$$\text{(I)}\quad \frac{1}{v} = \frac{K_M}{V_m} \cdot \frac{1}{S} + \frac{1}{V_m};$$

$$\text{(II)}\quad \frac{S}{v} = \frac{1}{V_m} \cdot S + \frac{K_M}{V_m};$$

$$\text{(III)}\quad V_m = v + \frac{v}{S} \cdot K_M.$$

A plot of the variables of such equations is commonly used for estimating the constants from a set of experimental data. Plots I and II were originally worked out by Lineweaver and Burk (*2*). They are used almost exclusively, whereas Plot III has been used only occasionally (e.g., [*3*]). Plot III, nevertheless, has great advantages over I and II. Since to the author's knowledge this has never been shown on a comparative basis, the importance of such plots in enzyme chemistry may warrant this note.

In Fig. 1, each curve represents the same theoretical case of an enzyme-substrate system with a K_M of 10^{-3} and a V_m of 100. The same points, representing equal increments of v, have been plotted according to Plots I, II, and III. In Plot I, the coordinates go to infinity when $S \rightarrow 0$, whereas in Plot II this is the case when $S \rightarrow \infty$. The half-maximum point (dotted lines) lies in both cases to one side of the curve. Experimental data that may have been obtained with about equal accuracy will be overemphasized at low values of v in Plot I and at high values of v in Plot II. The points at the other end of the curve are apt to be underestimated or even neglected. This is statistically unsound.

None of these objections holds for Plot III. Here the curve has a finite and positive intercept with both coordinates. The half-maximum point lies in the middle of the curve, and on both sides of this point equal emphasis is laid upon all data.

Equal emphasis upon all data is of special importance in cases where there is no certainty that only one enzyme is involved, as can be seen on the basis of Fig. 2. Here a system of two enzymes (A and B), acting simultaneously and independently on the same

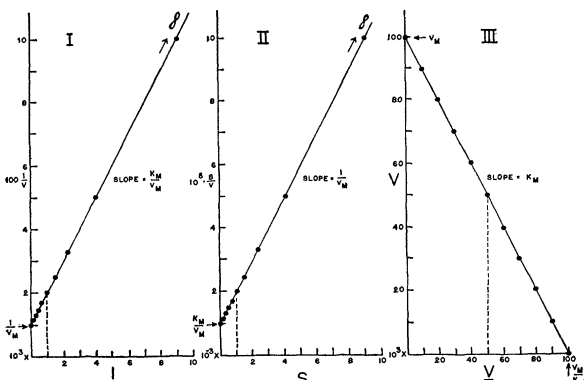

FIG. 1. The three possible linear plots of the Michaelis equation, representing a theoretical case of an enzyme system with $V_m = 100$ and $K_M = 10^{-3}$.

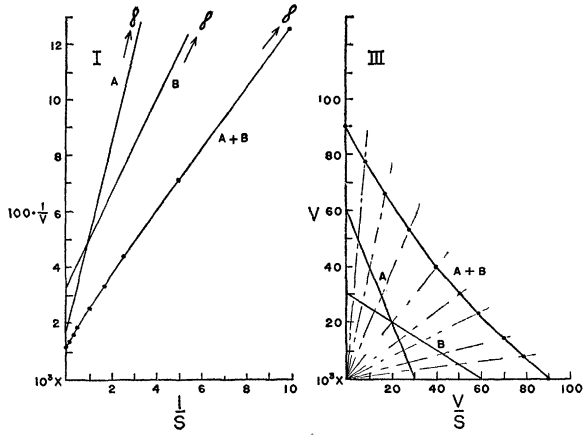

FIG. 2. Theoretical case of two enzymes, A and B, acting simultaneously and independently on the same substrate, with the constants different. The two plots correspond to I and III in Fig. 1. Only in Plot III the resulting line represents a simple addition (along the lines through the origin) of the individual plots.

substrate over a wide range of concentrations, is represented by Plot I and by Plot III. The difference in the constants of the two enzyme systems causes a curvature of the resulting lines. In Plot I, however, this curvature can be noticed only at one end of the curve. In the case of an actual experiment, where an experimental error is involved, the points might easily be taken as representing a straight line. The shorter the range of substrate concentrations applied, the better would be the fit. The corresponding constants could then quite erroneously be taken as the constants belonging to a single and hypothetical enzyme system. Similar difficulties would obtain if Plot II were used. The estimation of the constants will be reliable only if both Plot I and Plot II have been used with the same results, and a wide range of substrate concentrations has been applied.

In Plot III (Fig. 2), no straight line is simulated because enzymes A and B contribute to the curve in direct proportion to the magnitude of the constants. The coordinates of any point on the curve are Σv and $\Sigma \frac{v}{S}$, and the resulting curve is a simple addition of the two individual curves. A line through the origin represents values of v at a particular substrate concentration, and additions and subtractions must be made along such lines.

A situation similar to the theoretical case described above was encountered when a pancreas extract was used as the source of esterase (*4*).

In Plot III (Fig. 1), where V_m is directly given by the intercept with the ordinate, the intercept with the abscissa also has significance. Its value is given by $\lim_{S \rightarrow 0} \frac{v}{S} = \frac{V_m}{K_M}$. In an additive sense, v becomes negligible with respect to V_m at low substrate concentrations. From the original equation, it follows then that under such conditions v may be set equal to $\frac{V_m}{K_M} \cdot S$.

The intercept with the abscissa is therefore the reaction constant of the first order reaction that is approximated at low substrate concentrations.

Under physiological conditions, where the substrate concentration may be low, the reaction rate will be determined by $\frac{V_m}{K_M}$, in which expression $\frac{1}{K_M}$ is related to the affinity of the substrate for the enzyme. In this respect comparison of the rates at which two substrates are attacked has little meaning if only one arbitrarily chosen substrate concentration has been used. It is clear from the above that the complete concentration curves must be determined. If these curves cross, the situation at relatively high concentrations may be the reverse of that at low concentrations. In view of these considerations, the intercept with the abscissa, when Plot III is used, may give directly the most important constant of an enzyme system. In terms of Equations I and II, this intercept equals $\frac{k_3}{K_M}$ or $\frac{k_1 k_3}{k_2 + k_3}$ per amount of enzyme.

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Inhibition of Methylcholanthrene Carcinogenesis by Hypophysectomy[1]

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The significance of pituitary function in neoplastic disease is indicated by the many and diverse neoplasms that develop in the rat following the prolonged administration of growth hormone (*1–4*) and the absence of both growth hormone–induced and spontaneous tumors in the hypophysectomized rat (*5*). The present report is concerned with the inhibitory effect of hypophysectomy in methylcholanthrene carcinogenesis.

For this study 60 adult female rats of the Long-Evans strain were divided into four groups and treated as follows: (1) 15 rats were implanted with methylcholanthrene pellets into the right gastrocnemius muscle; (2) 15 rats were maintained without treatment as controls; (3) 15 rats were hypophysectomized; two weeks following hypophysectomy methylcholanthrene pellets were implanted into the right gastrocnemius muscle; (4) 15 rats were hypophysectomized and maintained without further treatment as controls. All animals were weighed and examined for tumors every 5 days for a maximum period of 316 days.

During the period of observation it was noted that 12 of the 15 rats treated with methylcholanthrene developed a palpable increase in connective tissue at the site of implantation as early as 3½ months and that 8 of these developed rapidly growing tumors after periods varying from 195 to 299 days following the implanting of methylcholanthrene. Prior to the completion of the experiment it was necessary to sacrifice 4 of these animals because of large tumors. Histologic examination of these tumors showed all of them to be sarcomas arising in skeletal muscle.

None of the normal controls developed comparable lesions.

Of the group of 15 hypophysectomized rats treated with methylcholanthrene, only one developed a sarcoma at the site of implantation. There was little or no connective tissue reaction to the methylcholanthrene pellet in the others. Nine of this group survived for more than 199 days, and of these there were 6 which survived for the total experimental period of 316 days.

There were no tumors in the hypophysectomized control group.

Although the numbers of animals used in these experiments are not great enough to warrant a definite conclusion at this time, nevertheless it is considered that the results are sufficiently important to deserve a preliminary note. Additional animals subjected to identical, as well as similar, conditions are now being investigated, following which studies a final report will be made.

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