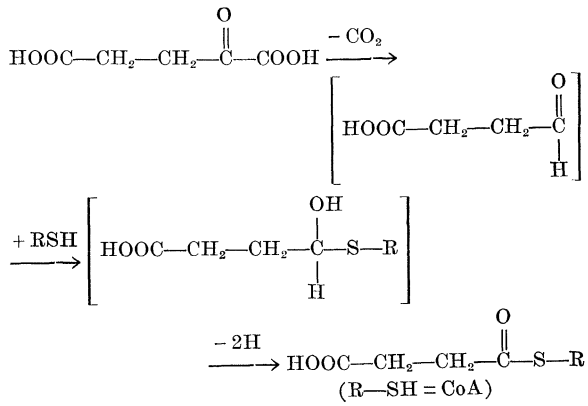
elution with aqueous pyridine, or by extraction with a phenol-benzyl alcohol mixture. The isolated sample reacted with sulfanilamide in the presence of crude pigeon liver acetone powder extract (*3*) under conditions where succinate, CoA, and cysteine were inactive. The hydroxamic acid derived from the sample has been identified by paper chromatography.

The equilibrium of the reaction is far to the right and is independent of pH. Triphosphopyridine nucleotide will not replace DPN in the reaction. Although pantetheine (synthetic LBF) (*11*) is inactive at low levels (1.3×10^{-4} *M*), high concentrations (6.7×10^{-3} *M*) can replace CoA. Protogen B,[5] α-lipoic acid,[5] and boiled extracts of the oxidase containing protogen in coenzyme form (*5*) are inactive.

From the data presented, both DPN and CoA may be considered as prosthetic groups of the α-ketoglutaric oxidase. The reaction mechanism in which the brackets refer to enzyme complexes may be represented as follows:



The initial decarboxylation has been indicated previously (*5*) and is further supported by the rapid incorporation of radioactive CO_2 into α-ketoglutarate in the absence of DPN or CoA (*12*).

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[5] Kindly supplied by T. H. Jukes and L. J. Read.

Direct Determination of Maximal Daily Metabolism of Alcohol

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Not long ago one of us (*1*) estimated that the maximal daily metabolism of alcohol by a man of average weight was represented by a quart of 100-proof liquor. This estimate was based on prolonged feeding experiments in dogs and the demonstrable similarity in the rate of alcohol metabolism in the dog and in man.

In spite of the simplicity of the direct experimental approach to this problem in man, search of the literature failed to reveal any objective information, and for this reason the present work was undertaken. Four subjects were used, three men and one woman. Two were moderate drinkers, and two had drunk to excess sufficiently to classify them as chronic alcoholics. They varied over a wide range as to age and body build. The experimental procedure was essentially the same in all cases. A test dose of 1.0 ml/kg of 95% alcohol diluted to about 20% with water was administered by mouth on an empty stomach, after which samples of venous blood were taken hourly and analyzed for alcohol by a modification of Winnick's method (*2*). From the length of time alcohol remained in the blood the amount metabolized per hour was calculated. Subsequently this amount was administered hourly, either as diluted alcohol or as an acceptable alcoholic beverage, and the blood or saliva alcohol concentration was determined at suitable intervals. To avoid undue inconvenience to the subject, during the night three doses three times the size of the hourly dose were given, which required only two awakenings. The results can best be presented for each individual subject.

Subject 1 was a moderate drinker, male, age 44, weight 61.4 kg, height 168 cm, body surface (Dubois) 1.70 m^2. The estimated hourly dose of 12.0 ml 95% alcohol resulted in 71 hr in a blood alcohol concentration of 17 mg %. Increasing the dose to 15.0 ml resulted in 48 hr in a rise to 159 mg %. According to Widmark's formula (*3*), it can be calculated that this rise in blood alcohol concentration would require 81 ml 95% alcohol; actually the subject received during this time 720 ml. This, less the amount required to produce the rise in blood alcohol concentration, amounts to 639 ml in 48 hr, or 320 ml daily.

Subject 2 was a moderate drinker, male, age 60, weight 65.0 kg, height 173 cm, body surface 1.79 m^2. The estimated hourly dose was 10.0 ml, which resulted in 54 hr in a blood alcohol of 8 mg %. Increasing the dose to 12.5 ml for 51 hr raised the blood alcohol to 91 mg %, which rise would require 52 ml alcohol. The actual amount given, 638 ml, less this excess, amounts to 586 ml in 51 hr, or 276 ml daily.

Subject 3 was a chronic alcoholic, female, age 32,

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.