

recorded in calories per kilo per hour, varied within $\pm 7.5\%$ of the average rate for that animal. The average basal metabolic rates of these normal animals varied, however, between 3.22 and 4.09 calories per kilo per hour.

In 8 guinea pigs weighing between 340 and 500 gm. we found that daily oral administration of KI in 0.1 gm., 0.05 gm., and 0.01 gm. doses, over a 25-day period caused a definite, although not very great, increase in the basal rate of metabolism in 2 animals; a variation within the upper range of the normal metabolic values in one animal; a variation falling in the lower range of the normal metabolic curve in one animal; and, in 4 animals, variations in the basal metabolic rate corresponding to the average variations found in the controls. Of the 2 animals which showed an increased caloric output, one showed an increase of approximately 20% above the normal range of values in the first 10 days; the other showed a rise of approximately 10% above the range of variation of the controls, which occurred between the tenth and twentieth days. The average basal metabolic rate of all the KI fed animals was slightly above the average of the 8 control animals. All animals gained in weight throughout the experiment. No correlation was to be noted between the doses of KI given and the changes in the basal metabolism.

Our results, therefore, agree essentially with those of Cordonnier. From our determinations, it would appear that in guinea pigs, as in rabbits, the effect of oral administration of KI on the basal metabolic rate may be variable, and is not very great. In our experiments, however, KI feeding in guinea pigs tended to cause a slight increase in the basal metabolic level, rather than a decrease.

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Titrimetric Measurement of Fermentation.

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The fact that yeast in high concentration ferments glucose very rapidly has been put to considerable use in the analysis of blood and other biochemical material. The fermentation can be so regulated that small quantities of glucose are completely broken down in a few minutes at room temperatures. The evolution of CO_2 takes

place so rapidly that the red color of phenol red (indicator) in a weakly alkaline glucose-yeast mixture turns to yellow in a few seconds.

In an attempt to apply this phenomenon as a means of quantitative observations in the study of fermentation processes, several serviceable procedures were tried out. The one to be described promises usefulness as the basis of a rather simple analytical technique.

The reagents employed are: (a) 0.01 molecular sodium carbonate solution, (b) 0.06% aqueous solution of phenol red, and (c) a 20% yeast suspension prepared by rubbing up 20 g. of commercial baker's yeast in water and making up the volume to 100 cc.

The procedure is as follows: Measure into a test tube 5 cc. of the sugar solution to be studied. Add one drop of phenol red and adjust the reaction to slight alkalinity (pink color, pH 7.2 to 7.4). Introduce into each of 2 other test tubes 5 cc. of the yeast suspension and 2 drops of phenol red, and add dropwise of the standard sodium carbonate solution until a distinct pink color persists for at least 30 seconds. To one of the yeast tubes 5 cc. of neutral (or neutralized) water are added; this serves as blank. The content of the other yeast tube is transferred into the tube containing the sugar, and the 2 fluids are immediately mixed by inversion of the stoppered tube. The pink color begins to fade in a few seconds, and at this point the titration must begin. A few drops at a time of the standard sodium carbonate solution are run in from a burette and mixed with the fluid by inversion of the tube. The rate of the titration must be so conducted as to keep the color of the indicator near the initial pink shade, and allow it at no time during the procedure to turn yellow. The end of the titration is indicated by the persistence of the initial pink shade for no less than 30 seconds after the last addition of sodium carbonate.

Meanwhile the color in the blank yeast has faded somewhat owing to the CO_2 produced by the respiration of the yeast. This is now titrated until the original pink color is restored. Deduction of this blank titration from the first titration figure, gives the titration value corresponding to the carbon dioxide derived from the sugar alone.

The procedure as outlined is but an example of the application of a rather flexible method, alterable to suit various experimental conditions and aims. With its aid it is possible to study the effect of a number of factors (concentration of yeast and of sugar, the sugar/yeast ratio, temperature, etc.) with greater facility and rapidity than is afforded by either the gasometric measurement of carbon dioxide or the usual methods of sugar determination.

TABLE I.
Amount of 0.01 Molar Na_2CO_3 Solution Consumed in the Titration of CO_2 Formed When 5 cc. of Glucose Solution Are Fermented with 5 cc. of 20% Yeast Suspension.

1	2	3	4	5
Amount of glucose	Time used for titration	CO_2 formed in yeast-glucose mixture	0.01 molar Na_2CO_3 solution consumed for titration of CO_2 formed in yeast suspension without glucose	CO_2 formed from glucose (Column 3 minus col. 4)
<i>mg.</i>	<i>minutes</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>
5	5-6	0.46	0.05	0.41
10	6-7	0.85	0.05	0.80
15	8-10	1.26	0.06	1.20
20	9-11	1.67	0.07	1.60
25	11-12	2.11	0.07	2.04

Table I is presented as an example of experiments carried out with our procedure. The results recorded, each representing the average of 3 closely agreeing titrations, show a linear proportionality between glucose concentration and titration figures, with yeast concentration, total volume and temperature kept unchanged. The CO_2 produced may be calculated from the equation

$$\text{pH} = 6.1 + \log \frac{[\text{BHCO}_3]}{[\text{free CO}_2]}$$

The value of BHCO_3 is known from the total amount of sodium carbonate introduced, the pH is indicated by the color of the mother liquor.

In possession of the titration equivalents of known amounts of fermentable sugars, it is possible to utilize this technique as an analytical procedure for the determination of sugars. In Table II are given some determinations of fermentable sugar in diabetic urine. Although we do not propose the use of this technique as an accu-

TABLE II.
Determination of Fermentable Sugar in Urine by a Fermentation-Titration Method.

No.	Shaffer-Hartmann method (modified)			Fermentation-titration method
	Total reduction	Non-fermentable reducing subst.	Fermentable sugar	
	%	%	%	%
1	1.42	0.16	1.26	1.26
2	2.64	0.17	2.47	2.30
3	0.79	0.21	0.58	0.58
4	4.66	0.11	4.55	4.58
5	0.47	0.08	0.39	0.38
6	1.31	0.19	1.12	1.20

rate quantitative method, it may become useful in the clinical laboratory, especially in cases where the distinction between glucose and non-fermentable sugars becomes necessary (lactosuria, pentosuria). The figures in Table II demonstrate that with a little practice fairly accurate results can be obtained. Since these results represent only fermentable sugar, for comparison the same was determined by the Shaffer-Hartmann method. Copper reduction methods in general furnish sugar values that are too high, since urine contains varying amounts of reducing substances other than sugar, thus the latter must be determined separately in the fermented urine and deducted from the total reduction in order to obtain the amount of fermentable sugar (glucose). It may be noted in the figures in Table II that by ignoring this fact and accepting total reduction values as sugar, the error inherent in copper reduction methods may exceed the experimental error attendant upon our simple semi-quantitative procedure.

The range of glucose concentrations recorded in Table I does not by any means represent the limits of applicability of this technique. It is effective with considerably higher as well as lower concentrations; in fact it is well possible to follow by titration with 0.001 molecular sodium carbonate the fermentation of as little as 0.5 mg. of glucose.