CXXVIII. STUDIES ON REVERSIBLE DEHYDROGENASE SYSTEMS.

I. THE REVERSIBILITY OF THE HYDROGENASE SYSTEM OF *BACT. COLI*[1].

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STEPHENSON AND STICKLAND [1931] described an enzyme in various bacteria which could catalyse the reduction of oxygen, methylene blue, nitrate, sulphate or fumarate by molecular hydrogen. Consideration of this catalysis discloses a striking resemblance between hydrogenase and platinum black. Both the enzyme and the heavy metal catalyse the reaction

$$H_2 \rightleftharpoons 2H \rightleftharpoons 2H^{\cdot} + 2\epsilon.$$

In the presence of platinum black, this reaction proceeds reversibly (*cf.* the use of platinised platinum electrodes for the measurement of p_H). The experiments which follow show that the same equilibrium point between molecular and ionic hydrogen is reached in the presence of *Bact. coli* as in the presence of platinum black.

EXPERIMENTAL METHODS.

Bact. coli was grown in Roux bottles on a broth agar in the usual fashion. After two washings by centrifuging, the thick mass of bacteria was treated with saturated toluene water at room temperature for 15 minutes. This treatment sufficed to eliminate completely all endogenous reducing power.

The equilibrium between H_2 and $H^{\cdot}$ was measured with methylviologen (dimethyl-$\gamma\gamma'$-dipyridylium chloride), an indicator which lies in the range of the hydrogen electrode between p_H 7 and 9. The indicator was prepared according to the directions of Michaelis and Hill [1933], and the recrystallised chloride gave theoretical figures on elementary analysis.

The equilibrium potential was measured both colorimetrically and electrometrically. The colorimetric technique consisted essentially in setting up Thunberg tubes each containing buffer, bacterial suspension and methylviologen. The tubes were evacuated thoroughly and filled with oxygen-free hydrogen at a definite partial pressure according to the method of Stephenson and Stickland [1931]. Since the reaction proceeds very rapidly at 40°, the tubes were incubated at that temperature for an hour with frequent vigorous shaking. Then the temperature was lowered to 30° for the final measurements, at least 2 hours being allowed for equilibrium to be reached. For estimation of the degree of reduction a set of standards was prepared containing various fractions of the

[1] A preliminary note has already appeared in *Nature* (1934), **133**, 573.

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total amount of indicator solution used in the experimental tubes. Both the total volume of fluid and the volume of bacterial suspension were the same in all the standard and experimental tubes. Reduction of the indicator in the standards was effected by adding a few crystals of sodium hydrosulphite and an excess of solid sodium carbonate.

The electrode vessels for the electrometric measurement of the equilibrium were constructed according to the design of Borsook and Schott [1931]. The only technical difficulty was the preparation of an agar bridge which could withstand a vacuum. By elimination of undissolved particles and air bubbles from the molten agar, bridges could be made to withstand a pressure of one atmosphere for the duration of several days, but the reliability rarely extended beyond the first few experiments.

The setting up of the experiment in the electrode vessel was identical with that described for the colorimetric experiments. Six electrode tubes were mounted on a special rack in an air-thermostat maintained at 30°. This rack permitted of vigorous shaking of the tubes. All the six tubes dipped into a large beaker of saturated potassium chloride which made contact with a reference saturated calomel electrode.

The potentiometry is described in detail in a paper by Green [1933]. The presence of the bacteria renders the potential recorded at the electrode somewhat unsteady, and readings of the potentiometer can at best be correct to about 1 millivolt.

The p_H of the buffer solutions used was measured by the quinhydrone and hydrogen electrodes. The buffers were made up to a concentration of $M/5$, and the final p_H of the bacterial suspension was assumed to be identical with that of the buffer alone.

Experimental results.

If *Bact. coli* and colloidal palladium are equivalent as catalysts, then for the same p_H and partial pressure of hydrogen the extent of reduction of methylviologen should be the same whichever catalyst is used. Table I summarises the results of this comparison. One set of Thunberg tubes contained 2 ml. of $M/5$ buffer, 0·5 ml. of $M/1000$ methylviologen and 1 ml. of bacterial suspension. A duplicate set was prepared in the same way except that the bacterial suspension had been previously heated to 100° for 15 minutes to inactivate the hydrogenase completely, and a drop of colloidal palladium suspension had been added. It is

Table I.

		Degree of reduction (%)	
p_H	Pressure of H_2 (mm. of Hg)	*Bact. coli* as catalyst	Palladium as catalyst
7·32	498	29	28
7·32	200	24	22
7·83	199	37	37
7·83	81	26	27
8·33	80	51	51
8·33	29	37	40
8·96	30	56	56
8·96	10·5	45	45

clear that the equilibrium-point is identical in the two sets of tubes. These measurements represent the average of comparisons carried out by two independent observers, and with the exception of one reading the two sets of values agreed to within 5 %.

If the potentials corresponding to the observed degree of reduction in Table I are calculated from the E_0 of the indicator, a uniform discrepancy of about 20 millivolts is found between the observed and theoretical potentials for the system $H_2 \rightleftharpoons 2H^{\cdot} + 2\epsilon$. This can only mean that the assumption of Michaelis and Hill [1933] that the E_0 of methylviologen (−0·446 v.) is independent of p_H does not hold below p_H 9. Actually the measurements of these authors were confined to fairly alkaline p_H values, and their value for the neutral range is simply an extrapolation. One of us (D. E. G.) has noticed a similar discrepancy between the colorimetric and potentiometric values for the xanthine oxidase system in presence of methylviologen.

Table II contains a summary of the potentiometric measurements of the equilibrium $H_2 \rightleftharpoons 2H^{\cdot} + 2\epsilon$ in presence of *Bact. coli* and methylviologen, with

Table II.

p_H	Pressure of H_2 (mm. of Hg)	Potential (volts)		
		Observed	Theoretical	Difference
7·02	600	−0·401	−0·408	+0·007
7·71	100	−0·433	−0·439	+0·006
7·71	306	−0·448	−0·453	+0·005
7·71	428	−0·455	−0·458	+0·003
8·12	49	−0·452	−0·455	+0·003
8·12	109	−0·463	−0·464	+0·001
8·12	161	−0·470	−0·470	0·000
8·12	237	−0·477	−0·475	−0·002
8·12	598	−0·489	−0·487	−0·002
8·23	105	−0·464	−0·470	+0·006
8·23	138	−0·475	−0·473	−0·002
9·10	20	−0·484	−0·487	+0·003

variation of the hydrogen and hydrogen ion concentrations. The remarkable agreement between the observed values of the potential and those calculated from the hydrogen electrode equation

$$E_h = -\frac{RT}{F} p_H - \frac{RT}{2F} \log P_{H_2},$$

gives adequate proof that hydrogenase functions as a perfect catalyst, *i.e.* it increases the rate of attainment of the equilibrium but does not alter the equilibrium-point.

SUMMARY.

Bact. coli has been shown to catalyse the reaction

$$H_2 \rightleftharpoons 2H^{\cdot} + 2\epsilon$$

in a completely reversible way. The hydrogenase system is the most negative reversible oxidation-reduction system as yet described in living cells.

REFERENCES.

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