negative cysteine might be expected. The oxidation of cysteine is very complex, however (cf. Remick, 1943; Borsook *et al.* 1937), and E'_0 values at pH 7 as high as +0·06 V have been recorded (Williams & Drissen, 1930).

It is possible that adrenaline may be oxidized in living tissues by non-enzymic mechanisms such as those described above. The importance of adrenochrome has been stressed by Veer (1942*a*), who brought forward evidence that it is biologically active, and that it is a melanin precursor *in vivo*. The closely related pigment hallachrome was found by Friedheim (1932, 1933) to increase the oxygen consumption of erythrocytes, and probably plays a role in the maturation of reticulocytes (Gad, Jacobsen & Plum, 1944). Veer (1942*b*) obtained evidence for somewhat similar action of adrenochrome, in that it augments the leucocyte migration of animal bone-marrow transplantations *in vitro*.

The catalysis by adrenochrome of adrenaline oxidation is analogous to the non-enzymic step in the oxidation of tyrosine discussed by Kertész (1948), in that in each case the catalyst is an *o*-quinone. Leucoadrenochrome, however, is very rapidly autoxidized at pH 7, while dihydroxyphenylalanine oxidation is rapid only when catalyzed enzymically.

SUMMARY

1. The oxidation of adrenaline by atmospheric oxygen at pH 7 is catalyzed peroxidatively by cytochrome *c* and by methaemoglobin. Traces of hydrogen peroxide originally formed by autoxidation of adrenaline are later augmented by autoxidation of leucoadrenochrome.

2. The oxidation of adrenaline is catalyzed by adrenochrome, which acts as a hydrogen carrier.

3. Adrenochrome acts as a hydrogen carrier in catalyzing the oxidation of ascorbic acid by atmospheric oxygen. It does not affect the rate of oxidation of cysteine, catechol or quinol at pH 7.

4. The catalysis of the oxidation of ascorbic acid by 'oxidized adrenaline' solutions appears to be due to the adrenochrome content of these solutions.

Dr R. Lemberg is thanked for his guidance throughout the work, and Dr A. Albert for his continued interest. This work was financed by grants from the Wellcome Foundation and the National Health and Medical Research Council of Australia.

REFERENCES

Albert, A. & Falk, J. E. (1949). *Biochem. J.* **44**, 129.
Ball, E. G. & Chen, T. (1933). *J. biol. Chem.* **102**, 691.
Borsook, H., Ellis, E. L. & Huffman, H. M. (1937). *J. biol. Chem.* **117**, 281.
Borsook, H. & Keighley, G. (1933). *Proc. nat. Acad. Sci., Wash.*, **19**, 875.
Friedheim, E. A. H. (1932). *C.R. Soc. Biol., Paris*, **111**, 505.
Friedheim, E. A. H. (1933). *Biochem. Z.* **259**, 258.
Gad, I., Jacobsen, E. & Plum, C. M. (1944). *Acta physiol. scand.* **7**, 243.
Green, D. E. & Richter, D. (1937). *Biochem. J.* **31**, 596.
Hermann, H., Boss, M. B. & Friedenwald, J. S. (1946). *J. biol. Chem.* **164**, 773.
Kertész, D. (1948). *Enzymologia*, **12**, 254.
LuValle, J. E. & Weissberger, A. (1947). *J. Amer. chem. Soc.* **69**, 1567.
MacCarthy, C. L. (1945). Off. Pub. Bd., Report. PB47, Office of Technical Services, U.S. Dept. of Commerce.
MacCarthy, C. L. (1946). *Chim. Ind.* **55**, 435.
Randall, L. O. (1947). *J. biol. Chem.* **165**, 733.
Remick, A. E. (1943). *Electronic Interpretations of Organic Chemistry*, pp. 248–9. New York: Wiley and Sons.
Veer, W. L. C. (1942*a*). *Rec. Trav. chim. Pays-Bas*, **61**, 638.
Veer, W. L. C. (1942*b*). *Rec. Trav. chim. Pays-Bas*, **61**, 763.
Wiesner, K. (1942). *Biochem. Z.* **313**, 48.
Williams, J. W. & Drissen, E. M. (1930). *J. biol. Chem.* **87**, 441.

The Substrate Specificity of the Tyrosine Decarboxylase of *Streptococcus faecalis*

By G. H. SLOANE-STANLEY, *Department of Pharmacology, University of Oxford*

(*Received* 6 *August* 1948)

Streptococcus faecalis has been shown to possess an enzyme which decarboxylates L-tyrosine and L-3:4-dihydroxyphenylalanine, with the formation of carbon dioxide and the corresponding amines; this enzyme is known as tyrosine decarboxylase (Gale, 1940; Epps, 1944). L-3:4-Dihydroxyphenylalanine is also a substrate of the DOPA decarboxylase of mammalian tissues. This enzyme attacks neither L-tyrosine nor L-phenylalanine (Blaschko, 1939), but it does decarboxylate L-2:5-dihydroxyphenylalanine, L-*m*-hydroxyphenylalanine and L-*o*-hydroxyphenylalanine (Blaschko & Sloane-Stanley, 1948; Blaschko, 1949). The experiments described in this paper were done in order to find out whether 2:5-dihydroxyphenylalanine, *m*-hydroxyphenylalanine and *o*-hydroxyphenylalanine are substrates of the streptococcal tyrosine decarboxylase.

METHODS

Preparation of the enzyme. A strain of *Strep. faecalis* R (American Type Culture Collection no. 8043), given by Prof. I. C. Gunsalus, was used. The organism was maintained as a stab culture in glucose-marmite agar, renewed every fortnight. For experimental purposes it was grown in a liquid medium. Two media were used: a vitamin B_6-free medium and a medium containing pyridoxal; the latter was prepared by adding 500 μg. of pyridoxal (Merck) to each litre of the vitamin B_6-free medium. The vitamin B_6-free medium was similar to that developed by Bellamy & Gunsalus (1945); its composition is given in Table 1. The concentration of K_2HPO_4 in the medium was double that given by Bellamy & Gunsalus; this change approximately trebled the yield of tyrosine decarboxylase.

Table 1. *Medium used for growth of* Streptococcus faecalis *R*

(Quantities for 1 l. of solution; adjusted to pH 7·2–7·3 before autoclaving.)

Casein hydrolysate, dried, vitamin-free	10 g.
Anhydrous glucose	10 g.
K_2HPO_4	10 g.
Sodium acetate (hydrated)	2 g.
Salts *B* (Landy & Dicken, 1942)	5 ml.
DL-Alanine	200 mg.
L-Cystine	200 mg.
DL-Tryptophan	100 mg.
Thioglycollic acid	100 mg.
L-Tyrosine	100 mg.
Adenine sulphate	5 mg.
Guanine hydrochloride	5 mg.
Uracil	5 mg.
Nicotinic acid	5 mg.
Calcium *dextro*pantothenate	1 mg.
Riboflavin	1 mg.
Pteroylglutamic acid	44 μg.
Biotin	1 μg.

Before it was grown in either of the experimental media, the organism was subcultured through a liquid medium prepared by adding 0·1 μg. of pyridoxal to 9 ml. of the vitamin B_6-free medium. After inoculation from the stab culture, this medium was incubated for 24 hr. at 37°. The cell suspension was then diluted 20 times with sterile distilled water, and each 9 ml. of experimental medium was inoculated with 0·1 ml. of the diluted suspension.

Washed suspensions of intact resting cells were prepared as follows. Tubes, each containing 9 ml. of medium, were inoculated and incubated for 24 hr. at 37°. The cells were then centrifuged out and washed with 0·9% (w/v) NaCl; for the manometric experiments the cells from each tube were suspended in 1·5 ml. of distilled water.

Dried cells of the organism were prepared as follows. For each sample a flask containing about 400 ml. of warmed medium (with or without added pyridoxal) was inoculated and incubated at 37°. In the absence of pyridoxal the highest yields of tyrosine apodecarboxylase were obtained after 20 hr. incubation; in the presence of pyridoxal the best time of incubation was *c.* 14 hr. The flask was then cooled to 0°; the cells were centrifuged out, washed with ice-cold 0·9% (w/v) NaCl and suspended in a little ice-cold distilled water. The suspension was poured into *c.* 7 vol. of ice-cold dry acetone; the cells were then filtered off on a small Buchner funnel (using Whatman no. 50 paper), washed with small portions of ice-cold dry acetone, and finally dried *in vacuo* over H_2SO_4. About 250 mg. of dried cells were usually obtained from each 400 ml. of medium. They were stored *in vacuo* over H_2SO_4 at 0°.

Manometric procedure. In all experiments Warburg manometers were used, with conical flasks with one side bulb. The side bulb of each flask contained 0·4 ml. of substrate solution, except in substrate competition experiments, in each of which the side bulb of one flask contained 0·4 ml. of a solution of the amino-acid to be tested plus 0·4 ml. of 0·04 M-L-tyrosine suspension. The temperature of the bath was 28·5°. In the experiments with intact cells the gas phase was air, except in the studies with 2:5-dihydroxyphenylalanine when N_2 was used. In all experiments with dried cells the gas phase was N_2.

The contents of the main compartments of the manometer flasks depended on the cell preparation used. In the experiments with intact cells grown in the vitamin B_6-free medium, the main compartments contained: 0·5 ml. of cell suspension, 1·0 ml. of 0·075 M-phthalate buffer (pH 5·0), 1·0 ml. of pyridoxal hydrochloride solution (containing 10 μg. of pyridoxal) and 0·1 ml. of distilled water. Under these conditions the tyrosine apodecarboxylase in the cells is saturated with codecarboxylase formed within the cells from the added pyridoxal (Gunsalus & Bellamy, 1944). In these experiments the rate of evolution of CO_2 was low in the first few minutes after tipping but subsequently increased, and became steady after about 15 min.

In the experiments with dried cells grown in the vitamin B_6-free medium, the main compartment of each manometer flask contained: 1 mg. of dried cells (added as a suspension in distilled water), 0·2 ml. of M-acetate buffer (pH 5·5), 1·0 ml. of pyridoxal hydrochloride solution (containing 10 μg. of pyridoxal), 0·1 ml. of adenosinetriphosphate (ATP) solution (prepared from a sample of the barium salt, given by Boots Pure Drug Co. Ltd., and containing 1 mg. of ATP); distilled water was added to make the total volume of liquid, including that in the side bulb, 3 ml. Under these conditions, the tyrosine apodecarboxylase in the dried cells is saturated with codecarboxylase formed from the added pyridoxal and ATP by an enzyme in the cells (Umbreit, Bellamy & Gunsalus, 1945). With dried cells, the rate of evolution of CO_2 became steady within 5 min. after tipping.

In the experiments with dried cells grown in the medium containing pyridoxal (500 μg./l.), the main compartment of each flask contained: 10 mg. of dried cells (added as a suspension in distilled water), 0·2 ml. of M-acetate buffer (pH 5·5) and distilled water to make the volume 1·1 ml. The dried cells used for these experiments contained tyrosine decarboxylase already saturated with codecarboxylase. These cells will be referred to as 'complete' cells. In these experiments CO_2 was evolved at the maximum (steady) rate from the moment of tipping.

In every experiment, the steady rate of evolution of CO_2 was measured, and is given as V in the Tables; V_T represents the rate with 0·4 ml. of 0·04 M-L-tyrosine added as substrate.

RESULTS

Experiments with m-*hydroxyphenylalanine.* The results of some typical experiments are given in Table 2. Intact cells of *Strep. faecalis* R were

tested with two different concentrations of *m*-hydroxyphenylalanine: with the lower concentration no CO_2 was evolved (Exp. 1); with the higher concentration a small amount of CO_2 was apparently evolved, but it is doubtful whether this was outside the limits of experimental error (Exp. 2). With dried cells, however, *m*-hydroxyphenylalanine was definitely decarboxylated; the rate of reaction was about 30% of that with tyrosine as substrate.

Table 2. *Tyrosine decarboxylase of* Streptococcus faecalis *R, and* m-*hydroxyphenylalanine*

Exp. no.	Enzyme preparation	Conc. of DL-*m*-hydroxyphenylalanine (M)	V (μl. CO_2 evolved/hr.)	V_T (μl. CO_2 evolved/hr., tyrosine as substrate)	V/V_T
1	Intact vitamin B_6-free cells + pyridoxal	0·0027	0	300	0
2	Intact vitamin B_6-free cells + pyridoxal	0·014	10	490	0·02
3	Dried vitamin B_6-free cells + pyridoxal + ATP	0·011	220	810	0·27
4	Dried 'complete' cells grown with pyridoxal	0·022	360	1150	0·31

Substrate competition experiments suggested that tyrosine and *m*-hydroxyphenylalanine are both decarboxylated by the same enzyme. In one such experiment the rates of evolution of CO_2, in μl./hr., were: with 0·011 M-DL-*m*-hydroxyphenylalanine, 220; with 0·0053M-L-tyrosine, 810; with both amino-acids, 590.

The product of the decarboxylation of *m*-hydroxyphenylalanine is the corresponding amine, *m*-hydroxyphenylethylamine. This substance has sympathomimetic properties and raises the arterial blood pressure of the spinal cat (Barger & Dale, 1910; Blaschko, Holton & Sloane-Stanley, 1949). The liquid from manometer flasks in which *m*-hydroxyphenylalanine had been incubated with dried 'complete' cells of *Strep. faecalis* R was, therefore, injected into spinal cats whose arterial blood pressure was recorded; its pressor action was compared with that of synthetic *m*-hydroxyphenylethylamine. In one such experiment four manometer flasks were set up, each containing 10 mg. of dried 'complete' cells and 0·022M-DL-*m*-hydroxyphenylalanine in a total volume of 1·5 ml. They were shaken in the bath for 2 hr. after tipping, by the end of which time the evolution of CO_2 had almost ceased; the results of Exp. 4 were obtained from one of these flasks. The total volume of CO_2 which had been evolved in all four flasks was 1220 μl. The retention of CO_2 under these conditions had been found to be about 6% in a parallel experiment with tyrosine as substrate; therefore, the evolution of 1220 μl. of CO_2 corresponded to the formation of 1290 μl., equivalent to 8 mg., of *m*-hydroxyphenylethylamine. The liquid from all four manometer flasks was pipetted into a centrifuge tube; each flask was washed twice with 0·25 ml. of distilled water, and the washings were added to the tube. The mixture (8 ml.) was expected to contain 1 mg. of *m*-hydroxyphenylethylamine/ml. It was heated for 5 min. in boiling water and then centrifuged. Portions of the supernatant fluid were injected into the jugular vein of a spinal cat. Fig. 1 is a record of the arterial blood pressure and shows the results of the injection of: (*a*) 1·0 mg. of synthetic *m*-hydroxyphenylethylamine, (*b*) 1·0 ml. of the supernatant fluid, and (*c*) 0·7 mg. of synthetic *m*-hydroxyphenylethylamine. The injection of the supernatant fluid had an effect on the blood pressure similar to that of synthetic *m*-hydroxyphenylethylamine. The pressor response to 1·0 ml. of the supernatant fluid was slightly less than that of 1·0 mg. of the synthetic amine; this may have been due to the fall of the

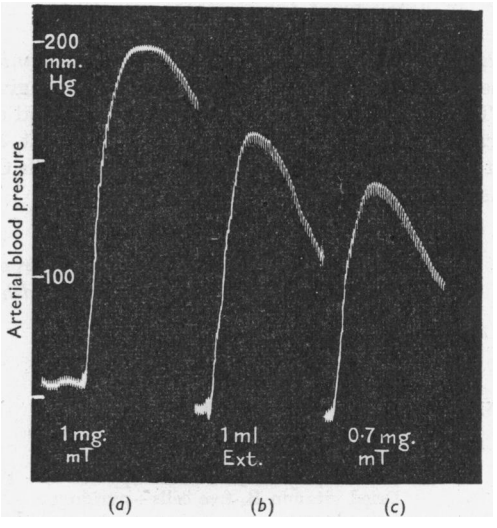

Fig. 1. Pressor action of *m*-hydroxyphenylethylamine. Arterial blood pressure of spinal cat. Tracing shows effects of intravenous injection of: (*a*) 1·0 mg. of synthetic *m*-hydroxyphenylethylamine; (*b*) 1·0 ml. of supernatant fluid from incubation of *m*-hydroxyphenylalanine with acetone-dried preparation of *Strep. faecalis* R, expected to contain 1·0 mg. of *m*-hydroxyphenylethylamine, and (*c*) 0·7 mg. of synthetic *m*-hydroxyphenylethylamine.

initial blood-pressure level between injections (*a*) and (*b*). The pressor effect of 1·0 ml. of the supernatant fluid was, however, greater than that of 0·7 mg. of the amine. These results show that, within the limits of error of this method, the concentration of *m*-hydroxyphenylethylamine in the supernatant fluid, as determined by pharmacological assay, agreed with that calculated to be present from the results of the manometric experiment. The total volume of CO_2 formed in this experiment, 1290 μl., was 43 % of that which would have been formed by the complete decarboxylation of the *m*-hydroxyphenylalanine added. This suggests that only one of the two optical isomers, probably the L-form, was attacked.

Experiments with o-hydroxyphenylalanine. DL-*o*-Hydroxyphenylalanine (0·0107 M) was incubated with dried vitamin B_6-free cells plus pyridoxal and ATP. CO_2 was evolved at a rate of *c.* 25 μl./hr.; with 0·0053 M-L-tyrosine as substrate, the rate of evolution of CO_2 was 810 μl./hr. In a substrate competition experiment done at the same time, the rate of evolution of CO_2 with both substrates was 775 μl./hr., which represented a decrease of 4 % in the rate of decarboxylation of tyrosine; in another, similar, experiment the decrease was 10 %. These decreases were probably not significant.

Experiments with 2:5-dihydroxyphenylalanine. The results of some typical experiments are given in Table 3. Intact cells of *Strep. faecalis* R did not decarboxylate 2:5-dihydroxyphenylalanine at all; but in some of the experiments with dried cells CO_2 was apparently evolved, but very slowly.

Table 3. *Tyrosine decarboxylase of* Streptococcus faecalis *R, and 2:5-dihydroxyphenylalanine*

Exp. no.	Enzyme preparation	Conc. of 2:5-dihydroxy-phenylalanine (M)	V (μl. CO_2 evolved/hr.)	V_T (μl. CO_2 evolved/hr., tyrosine as substrate)	V/V_T
5	Intact vitamin B_6-free cells + pyridoxal	0·011 (DL)	0	350	0
6	Intact vitamin B_6-free cells + pyridoxal	0·0053 (L)	0	380	0
7	Dried vitamin B_6-free cells + pyridoxal + ATP	0·0107 (DL)	20	810	0·025
8	Dried vitamin B_6-free cells + pyridoxal + ATP	0·0053 (L)	0	370	0
9	Dried 'complete' cells (grown with pyridoxal)	0·0107 (L)	15	490	0·03

In order to find out whether tyrosine decarboxylase can combine with 2:5-dihydroxyphenylalanine, substrate competition experiments were done. In Exp. 7, a mixture of 0·4 ml. of 0·08 M-DL-2:5-dihydroxyphenylalanine plus 0·4 ml. of 0·04 M-L-tyrosine was added as substrate; the rate of evolution of CO_2 was 590 μl./hr.; thus the addition of 2:5-dihydroxyphenylalanine had decreased the rate of decarboxylation of tyrosine by 27 % ($V_T = 810$). In another experiment the addition of 0·4 ml. of 0·04 M-L-2:5-dihydroxyphenylalanine decreased the rate of decarboxylation of the same amount of L-tyrosine by only 8 % (from 370 to 340 μl. of CO_2/hr.).

DISCUSSION

The results of the experiments with the isomers of tyrosine have shown that the rates of decarboxylation of these amino-acids by dried preparations of *Strep. faecalis* R decrease in the order tyrosine, *m*-hydroxyphenylalanine, *o*-hydroxyphenylalanine. The meta compound was decarboxylated by acetone-dried preparations of the organism at about 30 % of the rate observed with tyrosine; *m*-hydroxyphenylethylamine was formed in this reaction, as shown by its effect on the arterial blood pressure of the spinal cat. Substrate competition experiments suggested that both tyrosine and *m*-hydroxyphenylalanine were decarboxylated by the same enzyme. With *o*-hydroxyphenylalanine CO_2 was evolved so slowly that it is doubtful whether this amino-acid was decarboxylated at all. In substrate competition experiments it caused negligible decreases in the rate of decarboxylation of tyrosine; its affinity for tyrosine decarboxylase must, therefore, be very low.

In the experiments with *m*-hydroxyphenylalanine no CO_2 was evolved in the presence of washed suspensions of intact resting cells of the organism; as a result of this observation it was stated that the tyrosine decarboxylase of *Strep. faecalis* does not attack *m*-hydroxyphenylalanine (Blaschko & Sloane-Stanley, 1948). But the experiments described in this paper have shown that although this amino-acid is not decarboxylated by intact cells of *Strep. faecalis* R, it is attacked by acetone-dried preparations of the organism. This difference is interesting for it suggests that the undamaged cell membranes of the intact cells are impermeable to *m*-hydroxyphenylalanine, although they are freely permeable to the isomeric tyrosine.

It is not certain whether 2:5-dihydroxyphenylalanine is a substrate of tyrosine decarboxylase, or

can combine with the enzyme. It was apparently decarboxylated very slowly by acetone-dried preparations of *Strep. faecalis* R, and it caused slight decreases in the rates of decarboxylation of tyrosine by these preparations; but these effects may have been within the limits of experimental error.

As a result of the experiments of Epps (1944) it is evident that the tyrosine decarboxylase of *Strep. faecalis* attacks both tyrosine and 3:4-dihydroxyphenylalanine. The experiments described in the present paper have not been done with pure preparations of the enzyme, but the evidence presented suggests that *m*-hydroxyphenylalanine is in fact a substrate of tyrosine decarboxylase. It is evident that the replacement of the para hydroxyl group of tyrosine by a hydroxyl group in the meta position relative to the side chain produces a substance which is still decarboxylated by *Strep. faecalis*; on the other hand, our experiments with *o*-hydroxyphenylalanine have shown that the rate of decarboxylation of this compound by tyrosine decarboxylase is very low.

SUMMARY

1. Acetone-dried preparations of *Strep. faecalis* R decarboxylate *m*-hydroxyphenylalanine at about 30% of the rate at which they decarboxylate tyrosine. The reaction appears to be specific for the L-form of *m*-hydroxyphenylalanine, which is decarboxylated quantitatively to *m*-hydroxyphenylethylamine. Washed suspensions of intact resting cells of the organism have no detectable action on *m*-hydroxyphenylalanine.

2. Dried preparations of *Strep. faecalis* R do not decarboxylate *o*-hydroxyphenylalanine at a significant rate, nor has this amino-acid any detectable affinity for tyrosine decarboxylase.

3. Neither dried preparations nor intact cells of *Strep. faecalis* R have any significant action on 2:5-dihydroxyphenylalanine.

I wish to thank Dr H. Blaschko for his help and encouragement, and Mrs P. Holton for carrying out the pharmacological assays; I am grateful to the Medical Research Council for a Research Training Grant.

REFERENCES

Barger, G. & Dale, H. H. (1910). *J. Physiol.* **41**, 19.
Bellamy, W. D. & Gunsalus, I. C. (1945). *J. Bact.* **50**, 95.
Blaschko, H. (1939). *J. Physiol.* **96**, 50*P*.
Blaschko, H. (1949). *Biochem. J.* **44**, 268.
Blaschko, H., Holton, P. & Sloane-Stanley, G. H. (1949). *J. Physiol.* In the Press.
Blaschko, H. & Sloane-Stanley, G. H. (1948). *Biochem. J.* **42**, iii.
Epps, H. M. R. (1944). *Biochem. J.* **38**, 242.
Gale, E. F. (1940). *Biochem. J.* **34**, 846.
Gunsalus, I. C. & Bellamy, W. D. (1944). *J. biol. Chem.* **155**, 357.
Landy, M. & Dicken, D. M. (1942). *J. Lab. clin. Med.* **27**, 1086.
Umbreit, W. W., Bellamy, W. D. & Gunsalus, I. C. (1945). *Arch. Biochem.* **7**, 185.

The Effects of Applied Pressure on Secretion by Isolated Amphibian Gastric Mucosa

By R. E. DAVIES and C. TERNER, *Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, University of Sheffield*

(*Received* 20 *August* 1948)

Ludwig (1851) has shown that secretion by the submaxillary gland can occur against a pressure greater than the arterial blood pressure, but no evidence is available about the relation between pressure and secretion in the stomach. The apparatus described in this paper was developed to study simultaneously the rates of fluid secretion and respiration by isolated gastric mucosa. The results show that the secretion of hydrochloric acid can occur against pressure. This supports the view that the hydrostatic pressure of the blood is not an essential part of the mechanism of secretion of the solution of hydrochloric acid by the oxyntic cell.

EXPERIMENTAL

Apparatus

The apparatus used is shown in Figs. 1 and 2. It consists of a manometer *A*, a vessel *B*, and a hollow stopper *S*. *A* is a constant pressure manometer (modified from Dixon, 1943). Like a Warburg manometer it consists of a U-tube provided with a rubber reservoir R_1 containing modified Brodie solution. A graduated 0·5 ml. pipette *P* is attached to its right-hand limb; a rubber reservoir R_2 containing mercury is attached to the end of the pipette. The vessel *B* is a conical cup (volume *c.* 27 ml.) with a central ground socket J_1 and two side arms C_1 and C_2. The vessel is attached to the manometer by the ground joint J_2 on the side arm C_1. The side arm C_2, which is not shown in Fig. 2, has a tap