escaped into the vacuum system and the water level was restored. It was necessary to cool the cell during electrolysis and for this purpose it was wound with heavy-gauge copper wire, the ends of which dipped into an ethanol-CO₂ bath. As this cooling was still insufficient, the cold ethanol was splashed over the cell with a small brush. With a current of 0.11 amp., 1.6 ml. of ¹⁸O₂ at N.T.P. were evolved in 5 min.

When electrolysis was complete, the oxygen was expanded into the Toepler pump (*H*) with stopcocks *E* and *F* opened. With stopcock *E* closed it was then pumped into a previously evacuated apparatus, attached to the vacuum line through the standard joint *G* (B10). With water of 0.575 atom % excess ¹⁸O in the cell (determined by equili-

bration with CO₂ and carbonic anhydrase as described by Bentley, 1948), oxygen gas of 0.579 atom % excess ¹⁸O was obtained.

SUMMARY

An apparatus is described in which quantities of the order of 1.5 ml. of water may be electrolysed, and the evolved oxygen (or hydrogen) collected in a vacuum system.

The author is indebted to Miss M. G. French for experimental assistance and to Mr G. Dickinson for assistance with mass spectrometric analyses.

REFERENCES

- Bentley, R. (1948). *Cold Spr. Harb. Sym. quant. Biol.* **13**, 11. Bentley, R. & Neuberger, A. (1949). *Biochem. J.* **45**, 584.

Growth Factors for *Corynebacterium Diphtheriae*

4. PREPARATION FROM YEAST

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(Received 15 February 1949)

In an earlier paper, Chattaway, Happold & Sandford (1944) showed that certain gravis (and intermedius) strains of *Corynebacterium diphtheriae* required an unidentified growth factor when cultivated on a medium of known composition containing pimelic, nicotinic and pantothenic acids (Mueller, 1937*a, b*; Evans, Handley & Happold, 1939*a, b*). This factor, present in liver, was shown to be dialysable and resistant to proteolytic enzymes. Its properties were consistent with the presence in its molecule of both acid and basic groups probably in the form of an α -amino-acid component, since the activity was destroyed by ninhydrin.

The factor appeared to have no direct connexion with any of the unidentified growth factors for *Lactobacillus casei*, cf. Dolby, Happold & Sandford (1944), but showed some similarity to streptogenin, which was prepared by Woolley (1941) from various purified proteins, and is required by certain strains of *Streptococcus haemolyticus*. The present paper reports the separation and concentration from yeast of a number of factors similar in many respects to the liver factor of Chattaway *et al.* (1944).

EXPERIMENTAL

Method of microbiological assay

Organism. The strain of *C. diphtheriae* used for assessing the potency of preparations was a gravis strain, subtype Dundee (Barton). In all cases the growth was observed

in $6 \times \frac{5}{8}$ in. test tubes containing the medium outlined below.

Inocula. The strain was maintained on Löffler slopes in the refrigerator and subcultures were made on blood-agar plates. A sufficient amount of an 18 hr. subculture was introduced into 10 ml. of sterile water to produce a faint turbidity at the surface. This suspension was dispersed by shaking and one drop (approx. 0.03 ml.) used for inoculation.

Basal medium. The basal medium employed, shown in Table 1, differed from that described by Chattaway *et al.* (1944). Certain amino-acids hitherto omitted were included in order to bring its composition more nearly into line with a casein-hydrolysate medium on which the organism grows profusely. In the early experiments it was found that test cultures occasionally grew in the basal medium. This was traced to an impurity in the aspartic acid, which was then omitted without alteration in the response of the organism to the factor. Pimelic acid was also omitted since the organism was provided with preformed biotin.

Determination of growth. The organism used grows exclusively as a pellicle under the conditions of test, a fact which enables a visual assay of growth to be made, and which precludes all other methods with the exception of a determination of the N₂ content of a whole culture. This latter method was discarded since an adequate expression of growth could be made in terms of arbitrary degrees of pellicle formation. Ten degrees of growth were recognized, ranging from one given a numerical value of 4, corresponding to a heavy pellicle over the surface of the medium, through 3, 2, 1.5 and 1, at which value the whole surface of the medium was covered with a thin film of growth, down to degrees of growth given the numerical values 0.5, 0.2, 0.1, 0.05 and 0.02, the last being a just discernible trace. Growth was determined after 72 hr. incubation at 37°. This method

Table 1. *Basal medium for the growth of Corynebacterium diphtheriae* (Dundee)

(Quantities for 1 l.)			
	mg.		μg.
Glycine	100	Biotin	1
DL-Alanine	400	<i>p</i> -Aminobenzoic acid	50
L-Valine	700	Riboflavin	100
L-Leucine	500	Nicotinic acid	200
L-Isoleucine	100	Calcium pantothenate	100
L-Proline	400	Pyridoxine	600
L-Hydroxyproline	20		
DL-Phenylalanine	200	2M-sodium lactate (from B.D.H. A.R. lactic acid)	20 ml.
DL-Aspartic acid	400	Na ₂ HPO ₄ ·12H ₂ O	1 g.
DL-Glutamic acid hydrochloride	2200	K ₂ HPO ₄	0.5 g.
DL-Serine	200	FeSO ₄ ·7H ₂ O	160 μg.
L-Tyrosine	300	NaCl	9 g.
DL-Methionine	100	MgSO ₄ ·7H ₂ O	0.7 g.
D-Arginine	400		
L-Histidine (base)	400	Salts solution:	1.0 ml.
L-Lysine hydrochloride	600	CuSO ₄ ·5H ₂ O	0.05 g.
L-Tryptophan	400	ZnSO ₄ ·7H ₂ O	0.04 g.
L-Cystine	800	MnCl ₂ ·4H ₂ O	0.02 g.
DL-Threonine	50		

The medium was adjusted to pH 7.4 and 0.05 ml. of 0.5% CaCl₂/5 ml. of medium added aseptically after autoclaving.

Table 2. *The activity of yeast fractions as growth-promoting substances for Corynebacterium diphtheriae*

Fraction	<i>x</i> =	Concentration of additions (10 ^{-x} ml./ml. medium)									
		1	2	3	4	5	6	7	8	9	10
		(Activity numbers)									
Hydrolysate after removal of HCl (dry weight 250 mg./ml.)	17	100	17	1	0	0	—	—	—	—	—
Filtrate from norite	67	100	33	17	7	3	0	—	—	—	—
Filtrate after removal of inorganic material	33	100	100	67	50	33	7	0	—	—	—
After removal of lead as phosphate	0	17	67	100	67	33	17	1	0	—	—
Filtrate from phenylhydrazine containing some phenylhydrazine	0	0	100	100	67	50	33	17	7	—	—
Final extract after purification from phenylhydrazine and acetic acid (dry weight 170 mg./ml.)	33	67	100	100	100	100	100	100	67	33	17

has proved useful since several factors are capable of causing variation in the maximum growth obtained in a test and an approximate numerical expression of the results permits the values obtained in each series of tests to be expressed as a percentage of the maximum growth obtained at that time. These values, termed activity numbers, can then be compared in tests carried out at different times.

Preparation of active concentrate

Choice of material. Previous work had been carried out on liver residues from the preparation of the antipernicious anaemia factor (the factory treatment of which was unknown to us); later samples were found to be entirely without activity presumably due to some alteration in technique. Other sources, therefore, had to be explored. Both casein hydrolysates and yeast hydrolysates contain sufficient factor for growth of the organism, but it was decided to use yeast as large quantities were available.

Fresh brewer's yeast was usually employed, but one sample of dried baker's yeast showed similar activity when worked up in the same manner. Brewer's yeast was obtained within 24 hr. of draining and was not steamed; longer periods of standing resulted in autolysis and loss of activity. The active material is an essential growth factor for *Strep. faecalis* R and *Lb. casei* when these organisms are

grown on amino-acid media. Material has also been prepared from human urine which is active for the above strain of *C. diphtheriae*.

Preparation of active extracts. Fresh brewer's yeast (2 kg.) was made into a slurry with 8 l. of 2N-HCl, boiled rapidly for 1.5 hr., filtered, and the residue washed with 1 l. of water. PbO was added to the combined filtrate and washings to bring it to pH 2.8–3.0, the precipitate removed and the filtrate and washings shaken with charcoal (norite 200 g./l.) for 2 hr., and filtered. The norite was washed with water, the washings added to the filtrate and the pH adjusted to 6.5 with Na₂PO₄ in saturated solution. The filtrate, after the removal of lead phosphate, was concentrated to about 4 l. using capryl alcohol as an antifoam agent. To remove sugar impurities (mainly mannose) hydrated sodium acetate (2 kg.) and phenylhydrazine hydrochloride (200 g.) were added in 4 l. of water, and the phenylhydrazones filtered off 18 hr. later. Most of the excess phenylhydrazine was removed from the filtrate in the usual way with benzaldehyde (25 g.), the remainder by nine extractions with 1/3 vol. of ether. Acetic acid was removed by acidification with H₂SO₄ to pH 3, followed by repeated extraction with ether. The solution was then frozen repeatedly, and the successive crops of sodium sulphate crystals removed. The solution was concentrated in

vacuo below 40°, to about 1.2 l., treated with ethanol to a concentration of 40% (v/v), and the pH brought to 6.5 with baryta. The solution (Y 47) after removal of BaSO₄ had a dry weight of 170 mg./ml. and was active at 1.7×10^{-5} µg./ml. of medium. Table 2 gives the activities of fractions at various stages throughout the concentration.

Properties of yeast concentrate Y 47

Analytical figures are shown in Table 3. The preparation contained an appreciable quantity of free amino-acids, and also peptides, indicated by a positive biuret reaction. No histidine, tyrosine, arginine, tryptophan or free amines were present. Application of the reaction of Elson & Morgan (1933) showed the presence of material akin to amino sugars but not identical with glucosamine.

Table 3. *Analyses of yeast extract Y 47*

(Dry weight of Y 47 = 170 mg./ml.)

Material determined	Content (mg./100 mg. Y 47)
Total N (Kjeldahl)	6.26
NH ₃ -N	2.33
α-NH ₂ -N (ninhydrin)	0.92
S	Nil
Total P	0.39
Inorganic P	0.03
Carbohydrate as glucose (Hagedorn & Jensen)	53.2
Carbohydrate as glucose (Somogyi)	33.5

Inactivation. (a) *Hydrolysis.* Prolonged hydrolysis with 6N-HCl considerably reduced activity. Acid hydrolysis did not affect the α-amino N value appreciably.

(b) *Ninhydrin.* Refluxing Y 47 with 1/3 saturated KH₂PO₄ (20 ml.) and ninhydrin (500 mg.) for 15 min., removal of excess ninhydrin with alanine (225 mg.), refluxing for a further 15 min. and extraction of the pigments produced with CHCl₃ resulted in complete destruction of the active material.

(c) *Nitrous acid.* Treatment of Y 47 (0.8 ml.) with NaNO₂ (300 mg.) and 0.4N-HCl (10 ml.) overnight at room temperature resulted in complete loss of activity.

(d) *Oxazone formation.* The filtrate after removal of phenylhydrazones from Y 15 (5 ml.) by treatment in the cold with phenylhydrazine hydrochloride (0.4 g.) and hydrated sodium acetate (2 g.) was boiled with glacial acetic acid (1 ml.) for 15 min. This treatment resulted in a partial inactivation.

Identification of the active material of Y 47 with a number of ninhydrin-positive components

Many of the techniques, examined before the above scheme for separation of the active material from yeast was perfected, showed defects which proved of interest in the further study of the extract. Attempts to remove the considerable amounts of sugar present by the use of 80% ethanol, baryta, methanol, pyridine, dialysed iron, calcium chloride and basic lead acetate were all discarded owing to a partition of activity between the two phases, and extraction of Y 47 with *p*-cresol had a similar effect.

These results might be explained as the simple partition of a single factor, or by the presence of more than one factor. Attempts were therefore made to fractionate Y 47 to find if the activity was associated with one or more of the components.

The complete destruction of activity by treatment with ninhydrin suggested that the active components were amino-acids or peptides. Two-dimensional partition chromatograms of Y 47 on paper, using phenol and collidine, showed the presence of twelve ninhydrin-positive compounds (see Fig. 1). Of these, five have been identified, by their relative positions and by the similarity of their individual positions, with aspartic acid, glutamic acid, serine, glycine and isoleucine. The remainder which could not be correlated with known amino-acids have been assigned the symbols, S 1, S 2, S 3, P 1, P 2, P 3 and P 4. The two spots S 2 and S 3 were very weak, apparently indicating a low concentration, while P 2 was present in high concentration.

Chromatograms obtained after hydrolysis in 6N-HCl for 18 hr. at 120° in a sealed tube showed that P 1, P 2, P 3 and P 4 are peptide in nature, since they completely disappeared from the chromatogram. Under these conditions the spots, S 1, S 2 and S 3 were stable. This implies that they are either free amino-acids or acid-stable peptides. Partial acid hydrolysis (6N-HCl for 18 hr. at 100°) caused the breakdown of P 2 and P 4, but P 1 and P 3 were not destroyed. Indeed, the concentration of P 1 and P 3, as indicated by the intensity of the chromatogram spots, increased, suggesting the possibility that they are simpler peptides derived from P 2 and P 4. Similar degrees of stability were observed after hydrolysis with 5% w/v ammonia for 18 hr. at 100° in a sealed tube. Separation of these various amino-acids and peptides was undertaken before an investigation of their nature was attempted.

Separation by ionophoresis. The technique of silica-gel ionophoresis devised by Consden, Gordon & Martin (1947) for the separation of amino-acids and short-chain peptides was applied to Y 47. The gel was made from sodium silicate, 30 ml. of a 4N solution being brought to pH 7.4 (bromothymol blue) with H₃PO₄, and the whole diluted to 400 ml. This was poured into the trough and allowed to set. The cathode perfusate was 0.125N-NaH₂PO₄ and the anode perfusate 0.34N-Na₂PO₄ in the ratio of 1/7; 5 ml. samples of Y 47 were mixed with appropriate quantities of sodium silicate solution to make the filling for the gutter. The potential applied was generally 120 V., occasionally 60 V., and the length of the gel about 50 cm.

The electrode perfusion liquids were circulated continuously (cf. Hall, 1948), and fractions collected as they passed over the anode gel/liquid interface. After runs varying in length from 5 to 22 hr. the material had separated into six distinct bands. Tables 4 and 5 give the activity, composition and mobility of these bands. Most of the activity was associated with two bands, C 2/1, which moved rapidly towards the anode, and C 2/4 + C 2/5 which contain material remaining in or near the gutter.

Fig. 1. Phenol/collidine chromatograms of *Y 47* and its partial hydrolysate after heating at 100° in 6*N*-HCl for 18 hr. The size of the circles indicates approximately the concentration of the ninhydrin-positive substances, which are respectively: (1) *S*1; (2) aspartic acid; (3) glutamic acid; (4) serine; (5) *P*3; (6) glycine; (7) *P*1; (8) *P*2; (9) *S*3; (10) *S*2; (11) isoleucine; (12) *P*4.

Table 4. Separation of ninhydrin-positive components in *Y 47* by ionophoresis
(Separation in a silica gel, pH 7·4; potential 120 V.)

Direction of movement	No. of component	Distance travelled in 4 hr. (mm.)	Breadth of band after 4 hr. (mm.)	Composition determined by partition chromatography
Towards anode	<i>C</i> 2/1	70	24	<i>S</i> 1
	<i>C</i> 2/2	55	5	Glutamic acid, aspartic acid
	<i>C</i> 2/3	35	6	Serine
	<i>C</i> 2/4	15	14	<i>P</i> 1, <i>P</i> 2, <i>P</i> 4, glycine
Towards cathode	<i>C</i> 2/5	17	21	and isoleucine
	<i>C</i> 2/6	31	6	<i>S</i> 2

Table 5. The activity of six components of *Y 47* separated by ionophoresis
(*C* 2/4 and 5 also includes any material remaining in the gutter.)

Concentrations of additions, 10 ⁻² ml./ml. of medium (<i>x</i>)	<i>Y 47</i>	<i>C</i> 2/1	<i>C</i> 2/2	<i>C</i> 2/3	<i>C</i> 2/4 <i>C</i> 2/5	<i>C</i> 2/6
	(Activity numbers)					
1	100	0	0	0	67	0
2	100	1	0	0	100	17
3	100	67	17	33	67	0
4	100	67	33	17	67	1
5	100	33	33	1	33	1
6	100	17	17	1	17	0

A similar experiment with a streptogenin concentrate, kindly supplied by Dr D. W. Woolley (cf. Sprince & Woolley, 1945), showed no amino-acids even after prolonged

application of the potential except within a range of a few millimetres of the gutter. This is in agreement with other observations that streptogenin, although containing two of the peptides present in *Y 47*, does not contain any of the components moving more slowly in a phenol chromatogram, especially *S*1 (present in *C*2/1) and the free amino-acids glycine, serine, glutamic and aspartic acids.

Separation by partition chromatography. The technique devised by Dent (1947) for the separation of peptides from urine was used. Thirty-six spots of about 0·02 ml. each of *Y 47* were applied to a paper and chromatographed in phenol for 18 hr. The total length of the run was 342 mm. The two outside traces were removed and developed with ninhydrin and the main sheet cut accordingly into ten pieces. Of these, seven were initially judged to include all the ninhydrin-positive material present in *Y 47* while three, nos. 3, 5 and 9, represented the larger interspot spaces. The breadths of the bands and their composition as indicated by running subsequently in phenol or collidine are shown in Table 6.

1948) and phosphorylhydroxylysine (Gordon, 1948). Since serine is present in Y47, and S2, which has slight activity, gives a spot close to that of hydroxylysine, it seemed possible that S1 might be one or other of the two phosphoryl derivatives. Estimation of the inorganic P content of S1 before and after treatment with alkaline phosphatase showed that the material contained organic P, but no change occurred in the intensities of the spot for S1, or in the activity of the preparation, nor did any more spots appear.

Examination of P2. P2, the only other component yet obtained pure in sufficient amount, was hydrolysed by 6N-HCl at 120° for 18 hr. and shown to consist of a polypeptide containing serine, glycine and glutamic acid, while P1 was shown to be a partial hydrolysis product of this polypeptide.

Comparison of Y47 components with streptogenin

Examination of the streptogenin concentrate by means of a one-dimensional phenol chromatogram showed that it contained only three ninhydrin-positive materials (owing to the small amount available no two-dimensional chromatograms were made). Two of the spots were close to P1 and P2, while the third ran slightly faster than the complex containing P4, S2, S3 and isoleucine. Table 10 shows the activity of streptogenin compared with Y47. Direct comparison with the figures given in Table 7 for the peptides concerned is not possible, since during extraction of the peptides from the chromatograms changes occur in the salt concentration, and dry weight determinations are of no significance.

Table 10. *Comparison of the activity of Y47 and streptogenin in the growth of Corynebacterium diphtheriae*

Additions to medium, 10 ⁻² mg./ml. (x)	Streptogenin (Activity numbers)	Y47
2	100	100
3	50	100
4	17	100
5	0.1	100

Preliminary examination of synthetic peptides

The following dipeptides showed considerable activity at the concentrations (μ g./ml.) indicated: glutamylglutamic acid (0.01), glutamylglycine (0.03), glycylglutamic acid (0.1), leucylglutamic acid and glutamylalanine (from 5 to 10); alanylglutamic acid appeared to be inactive.

DISCUSSION

Several unidentified factors present in yeast extract or partial hydrolysates of protein have recently been described which appear to have some properties in common with the preparations described in this paper; the most notable being a factor required by certain strains of *Strep. lactis* (Smith, 1943), the

'sporogenes vitamin' of Peterson & Shull (1948), the factors required for maximum production of toxin by *Clostridium tetani* (Mueller & Miller, 1948) and streptogenin (Woolley, 1941). Peterson & Shull concluded from comparative assays of the two materials, using *Cl. sporogenes* and *Lb. casei* as test organisms, that their material and streptogenin were distinct. Since these two materials, and the yeast preparation described in this paper, contain a number of active components it is probable that they have one or more components in common; this is so for streptogenin and Y47. The potency of a given preparation for a given organism may also depend upon the relative concentrations of the different components.

The preparation of a concentrate from yeast extract having considerable activity for the growth of a gravis strain of *C. diphtheriae*, type Dundee, has shown among other things the difference between this fraction and one, with similar physiological properties, obtained from liver. The active material from liver, after such mild purification as extraction with ethanol and ether, and the removal of some material on fuller's earth, was highly adsorbed on charcoal (norite) at pH 3. Crude yeast extracts, however, could be treated with norite without any significant loss in activity; and although some degree of adsorption from the final concentrate occurred, it was never complete. The concentrates, of which Y47 is the most active so far obtained, are still mixtures of a variety of components, the activity being associated with two fractions (P1 and P2) which are probably peptide in nature and one (S1) which is stable to acid hydrolysis. The failure to extract or adsorb only the active material from Y47 compelled us to identify the components of the mixture and then separate these in turn.

Woolley (1946) has claimed that serylglucylglutamic acid has streptogenin activity of low order and that consequently it is either a portion of a more complex compound present in streptogenin concentrates, or is similar to, but not identical with, the active material. One of the peptides (P2), common to both the streptogenin concentrate and Y47, liberates serine, glycine and glutamic acid on hydrolysis. It is uncertain whether the amino-acids are joined in the order mentioned above, but the more stable peptide P1, formed by partial hydrolysis of the tripeptide, occupies the position on a chromatogram assigned to serylglucine or glutamine by Dent (1947); the latter, however, can be excluded on the grounds of stability. It is, therefore, probable that serylglucylglutamic acid has some effect on *C. diphtheriae* metabolism, but until P2 is obtained in larger amounts, and compared with a synthetic specimen of the tripeptide, it is impossible to be certain.

A number of synthetic glutamic acid peptides have been tested for activity, some of which induced

growth in our cultures of *C. diphtheriae*. The degree of activity was in no instance as great as that observed either with Y 47 or with the more active of its constituent factors. There is, of course, the possibility that other material having marked activity is present in these synthetic peptides at concentrations too low for chromatographic identification. The peptides P1 and P2, however, do not make up the whole of the activity for *C. diphtheriae*. S1, the slow-moving stable material, is destroyed by the action of ophio-L- α -amino-acid oxidase and hence is presumably an amino-acid. On the other hand, since according to Zeller & Maritz (1944, 1945) the presence of OH and COOH groups in amino-acids prevents attack by the oxidase, the position of S1 on chromatograms cannot be taken as indicating that it is a polycarboxylic or polyhydroxy amino-acid. Since this position is not altered after treatment with phosphatase, although inorganic phosphorus is produced, one must assume that the phosphate is liberated from some component giving no ninhydrin colour. The major part of the active material of Y 47 appears to be of a peptide or amino-acid nature, but there is some evidence for the existence of other active components as shown by the partial inactivation on osazone formation.

SUMMARY

1. The preparation is described of a concentrate from brewer's yeast which contains essential growth factors for certain strains of *Corynebacterium diphtheriae* gravis.

2. The activity of the whole preparation is associated with four of the twelve ninhydrin-positive components.

3. Most of the activity is associated with an amino-acid, S1, and a peptide, P1, while a second amino-acid, S2, and another peptide, P2, have slight activity.

4. The peptide P2 consists of serine, glycine and glutamic acid.

5. A number of synthetic glutamic acid peptides also have activity as growth factors for *C. diphtheriae*.

We wish to acknowledge the co-operation of Dr M. McMillan, in the early stages of this work, and to thank Messrs Joshua Tetley and Son Ltd. for supplies of yeast, Messrs Roche Products Ltd. for gifts of material and the Medical Research Council for a grant during the course of the work; and also to acknowledge the gift of a number of synthetic peptides from Dr R. Consden of the Wool Industries Research Association.

REFERENCES

- Chattaway, F. W., Happold, F. C. & Sandford, M. (1944). *Biochem. J.* **38**, 111.
 Consden, R., Gordon, A. H. & Martin, A. J. P. (1947). *Biochem. J.* **41**, 590.
 Dent, C. E. (1947). *Biochem. J.* **41**, 240.
 Dent, C. E. (1948). *Biochem. J.* **43**, 169.
 Dolby, D. E., Happold, F. C. & Sandford, M. (1944). *Nature, Lond.*, **153**, 619.
 Elson, L. A. & Morgan, W. T. J. (1933). *Biochem. J.* **27**, 1824.
 Evans, W. C., Handley, W. R. C. & Happold, F. C. (1939a). *Brit. J. exp. Path.* **20**, 41.
 Evans, W. C., Handley, W. R. C. & Happold, F. C. (1939b). *Brit. J. exp. Path.* **20**, 396.
 Gordon, A. H. (1948). *Nature, Lond.*, **162**, 778.
 Hall, D. A. (1948). *Nature, Lond.*, **162**, 105.
 Mueller, J. H. (1937a). *J. Bact.* **34**, 163.
 Mueller, J. H. (1937b). *J. Bact.* **34**, 429.
 Mueller, J. H. & Miller, P. A. (1948). *J. Bact.* **56**, 219.
 Peterson, W. H. & Shull, G. M. (1948). *Arch. Biochem.* **18**, 69.
 Smith, F. R. (1943). *J. Bact.* **46**, 369.
 Sprince, A. & Woolley, D. W. (1945). *J. Amer. chem. Soc.* **67**, 1734.
 Woolley, D. W. (1941). *J. exp. Med.* **73**, 487.
 Woolley, D. W. (1946). *J. biol. Chem.* **166**, 783.
 Zeller, E. A. & Maritz, A. (1944). *Helv. chim. Acta*, **27**, 1888.
 Zeller, E. A. & Maritz, A. (1945). *Helv. chim. Acta*, **28**, 365.

A New Manometric Method for Determination of Respiratory Quotients

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(Received 3 January 1949)

Manometric determinations of the respiratory quotient (R.Q.) require measurements of oxygen uptake, carbon dioxide production and, in the case of glycolysing cells, changes in the bicarbonate concentration of the medium. Several methods, due to Dickens & Simer (1930, 1931), Dixon & Keilin (1933), and Sumerson (1939), are available. Two identical,

or practically identical, samples of the biological material are needed when these methods are used. The initial bicarbonate concentration is obtained by acidifying and killing one sample at the beginning of the experiment, while the change in bicarbonate concentration is determined by acidifying the other sample at the end of the experiment. Intermediate