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A standard culture medium for general bacteriology

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INTRODUCTION

In this paper we propose to describe a general culture medium which can replace, or form a base for, many of the different culture media called for in routine bacteriology. In this laboratory over the past few years several methods of preparing routine media have been tried. The production of meat digests is expensive in time and labour and needs adequate analytical control to ensure uniform media; commercial dried media are relatively expensive and in our hands were not invariably reliable. The medium described here, which has been in use for more than 2 years, has proved to be both economical and reliable.

Table 1. *Composition of medium*

Casein digest (Oxoid Tryptone)	10 g.
Yeast autolysate (Marmite)	5 g.
Sodium glycerophosphate	10 g.
Potassium lactate (50 %, w/w)	10 ml.
Glucose	2 g.
Inorganic salts solution†	5 ml.
Water to	1000 ml.
Final pH 7·0–7·2	

† The inorganic salts solution contains: $MgSO_4.7H_2O$, 4·0 g.; $MnSO_4.4H_2O$, 0·4 g.; $FeSO_4.7H_2O$, 0·4 g.; water, 100 ml.; acidify with 2 drops of 10 N-H_2SO_4.

Our aim was to produce a good nutrient medium suitable for the growth of as wide a range of organisms as possible, yet cheap and easy to prepare, reproducible, and as fully defined as possible in terms of known nutritional factors. The medium we finally adopted is a modification of the CCY medium of Gladstone & Fildes (1940). It can be prepared and stored in concentrated form; from it by dilution and if necessary supplementation with one or two additional components, most of the standard media can be prepared. This provides a flexible system equally suitable for large or small users, and obviates the need for maintaining stocks of little used media which often deteriorate on storage.

COMPOSITION OF MEDIUM

The composition of the basal nutrient medium (CYLG = casein-yeast-lactate-glucose) is shown in Table 1. Some details of its chemical composition are shown in Table 2.

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Table 2. *Approximate content of some essential nutrients in CYLG medium*

General analysis (g./l.)	
Total N	1·67
Peptide and amino acid N	1·35
Free amino N	0·58
Purine N	0·014
Glucose	2·0 (0·011 M)
Lactate	4·5 (0·05 M)
Glycerophosphate	5·0 (0·03 M)

Inorganic (mg./l.)*	
Ca	6
Cl†	340
Cu	0·06
Fe	5·7
Mg	30
Mn	5
P	1180
K	2040
Na†	1670
S	46

Vitamins (mg./l.)‡	
Thiamine	0·15
Riboflavin	0·3
Nicotinic acid	3·0
Pyridoxine	0·175
Pantothenic acid	0·3
p-Aminobenzoic acid	0·03
Inositol	8·5
Choline	10·0
Biotin	0·005
Folic acid	0·065
Folinic acid	0·025
Vitamin B_{12}	0·05 μg.

	Amino acids (mg./l.)		
	From casein	From marmite	Total
Alanine	260	175	435
Arginine	350	90	440
Aspartic acid	550	170	720
Cystine	33	23	56
Glutamic acid	2000	240	2240
Glycine	175	85	260
Histidine	250	47	297
Isoleucine	550	110	660
Leucine	850	133	983
Lysine	690	137	827
Methionine	280	29	309
Phenylalanine	490	82	572
Proline	1000	86	1086
Serine	530	115	645
Threonine	380	110	490
Tryptophan	125	30	155
Tyrosine	530	72	602
Valine	620	123	743

* These do not include trace elements which may be present in glycerophosphate, lactate or glucose.

† The NaCl content of both casein digest and marmite may vary slightly from batch to batch.

‡ These figures only give the amounts contributed by marmite.

The following sources of information were used in calculating these figures:

(1) Society for General Microbiology Special Report (1956).
(2) Block & Weiss (1956).
(3) Information supplied by the makers of Oxoid Tryptone and Marmite.

Nitrogen source

Any general medium should supply adequate amounts of all the natural protein amino acids either free or in the form of small polypeptides. This is the main function of the various commercial 'peptones' sold as media constituents, but they vary widely in performance due to differences in starting materials and method of digestion (Society for General Microbiology, 1956). There are obvious advantages in a uniform source of protein as starting material, and enzymic digests of casein have proved very satisfactory (Leifson, 1943; Burnett, Pelczar & Conn, 1957); specifications for a good pancreatic digest of casein have been given (Burnett *et al.* 1957). Gladstone & Fildes (1940) used an acid hydrolysate of casein supplemented with a smaller amount of an enzymic digest of casein, but there would seem to be little advantage in this, even if the materials are all prepared in the laboratory. Good commercial enzymic digests of casein are available and we have used mainly Oxoid Tryptone (Oxo Ltd., Southwark Bridge Road, London, S.E. 1) which is light in colour and free from toxic impurities. The only amino acid in which casein is deficient is cystine; yeast autolysate also, however, contributes some cystine to the medium (Table 2) and we found no evidence of cystine deficiency for any of the organisms we tested, or any improvement in growth on adding more cystine or cysteine.

Yeast autolysate

The autolysis of yeast under controlled conditions yields a fairly uniform product which is a good source of growth factors and inorganic ions (Society for General Microbiology, 1956); such preparations will also contain amino acids, purines and pyrimidines and some carbohydrate. Yeast autolysates, marketed as food in the form of a thick paste, are readily available and are probably the most convenient and economical form to use. Of those we tested, marmite (Marmite Ltd., Seething Lane, London, E.C. 3) gave the best results. Storage at room temperature up to 2 years did not have any adverse effect on its nutrititive value in media.

Energy source and buffer

A mixture of amino acids provides a good energy source for many organisms, but some require, and the growth of many is improved by, other substrates. We have included both glucose and lactate. Utilization of glucose will, with many organisms, lead to acid production; utilization of lactate will tend to counteract this due to alkali production. To guard against any large changes in pH we have followed Gladstone & Fildes (1940) in including glycerophosphate buffer ($pK_2 = 6{\cdot}7$) in the form of sodium β-glycerophosphate ($Na_2C_3H_5(OH)_2PO_4 . 5\frac{1}{2}H_2O$; B.D.H.).

Inorganic ions

In most complex media no specific addition of inorganic ions is made; common media constituents usually supply adequate amounts of these (Society for General Microbiology, 1956). With our medium we found that growth of some organisms was improved by additional magnesium, iron or manganese, and we have added

these ions in the form of an inorganic salt solution. We have used potassium lactate (as the commercial 50 % (w/w) syrup) in preference to sodium lactate to provide a more balanced K/Na ratio, though we have no experimental evidence of its superiority.

PREPARATION AND USE OF MEDIUM

Preparation

To prepare the medium, weigh or measure all the constituents except glucose, dissolve in the appropriate volume of water, and filter. If glucose is added at this stage, some darkening will occur on autoclaving. We have found it preferable to autoclave the glucose as a separate 20 % (w/v) aqueous solution (at a pH not greater than 7) and add 10 ml. of this to each litre of sterile medium. We normally prepare the basal medium at ten times the normal strength (quantities as in Table 1 but with 100 ml. water and omitting glucose); the medium can be stored in this form at room temperature without autoclaving provided a volatile preservative is added; we add 3–4 ml. per litre of carbon tetrachloride/toluene (1:1, v/v).

Liquid CYLG medium is prepared from the concentrate by diluting it with 9 volumes of water, autoclaving and adding glucose.

For convenience in preparing agar media, we prepare stocks of double-strength agar (2·5 % New Zealand agar in water, filtered if necessary), distribute 100 ml. amounts in 200 ml. bottles (or other convenient quantities) and autoclave. CYLG agar is then prepared by adding to each 100 ml. double-strength agar 20 ml. concentrated medium and 80 ml. water, autoclaving, adding glucose, and distributing.

In preparing broth or agar media containing additional components, the supplements can be added when the concentrated medium is diluted.

Growth of bacteria

All organisms which grow well on meat digest media, such as enterobacteria, staphylococci and bacilli, grow equally well or in many cases better on CYLG broth or agar. In addition other organisms normally considered as requiring enriched or special media also grow well on the basal medium; among these are clostridia (incubated anaerobically), including both proteolytic and saccharolytic species and *Clostridium tetani*, streptococci, pneumococci, brucellae and lactobacilli. Neisseriae (including *Neisseria meningitidis* and *N. gonorrhoeae*) grow well in CYLG broth; for growth on solid media some means of counteracting the toxic effect of fatty acids is needed such as chocolate or serum agar or addition of starch or charcoal. Similar solid media are also suitable for growth of *Bordetella pertussis*.

Though the basal medium supports rapid and luxuriant growth of many organisms, they do not normally remain viable for long periods, and it is not suitable in this form as a storage or stock culture medium. An excellent medium for this purpose is obtained by preparing the medium at half strength either in the form of agar stabs (1·25–1·5 % agar) or as a semi-solid medium (0·5 % agar).

The basal medium is also unsuitable for the production of spores; a modification on which several species of bacilli and clostridia spored readily was obtained, by preparing the medium at one-quarter the normal strength.

MODIFIED AND SUPPLEMENTED MEDIA

Blood media

CYLG medium forms a good base for the preparation of all types of blood media.

Blood agar is prepared by adding blood to sterile basal agar; to 100 ml. double-strength agar add 20 ml. concentrated medium and 70 ml. water, autoclave and cool to 50° C.; then add 10 ml. blood and 2 ml. sterile 20 % glucose and pour plates. The medium gives typical haemolytic reactions with haemolytic organisms. The presence of glucose in blood media is normally considered undesirable, but the small amount present here improves growth of many organisms such as streptococci without interfering with the haemolytic reaction.

Chocolate agar (heated blood agar) is prepared in a similar manner except that the blood is added to hot agar medium and then heated at 80° C. for 5 min.

Serum broth and agar: add sterile serum to sterile CYLG broth or to sterile CYLG agar held at 50° to give a final concentration of 5 %.

Media for Haemophilus species

CYLG medium contains small amounts of haemin (from yeast autolysate) but no pyridine nucleotide coenzymes. It will therefore support slight growth of *Haemophilus canis* but not of other species unless supplemented with sources of these factors. Suitable conventional media are chocolate agar, heated blood broth (Alexander, 1958) and broth or agar supplemented with Fildes's peptic digest of blood (Fildes, 1920). While blood is a good source of haemin, its content of pyridine nucleotides will depend to a large extent on its freshness, and at best it is not a very good source (Schlenk, 1951) being greatly inferior to yeast; von Euler & Schlenk (1937) have pointed out that in terms of diphosphopyridine nucleotide (DPN) content 1 kg. yeast is equivalent to the blood of three horses. It therefore appears preferable to add pure haemin and DPN or the following preparations of haemin (at 1 % final concentration) and DPN-rich yeast extract (at 2 % final concentration).

Haemin. Centrifuge the red cells from 10 ml. blood and add to them 25 ml. acetone containing 0·3 ml. concn HCl, with shaking. Filter. Precipitate the haemin by adding 25–30 ml. water, filter and wash with water. The product (25–30 mg. crude haemin) is dissolved in 25 ml. 0·1 M-Na_2HPO_4 and autoclaved. This solution keeps well.

Yeast extract. Suspend 50 g. yeast in 100 ml. 0·2 M-KH_2PO_4 and heat at 80–85° C. for 20 min.; filter with a filter aid, or centrifuge, and sterilize the filtrate by Seitz filtration. Store in the cold or preferably frozen.

MacConkey media

MacConkey type media can be prepared from the concentrated base by adding a mixture of lactose, bile salts (or alternative inhibitors such as sodium dodecyl

sulphate) and neutral red in place of glucose. They appear to be fully comparable with the conventional media.

Anaerobic media

A medium comparable to the reinforced clostridial medium (RCM) of Hirsch & Grinsted (1954) can be prepared from CYLG medium by adding agar and a reducing agent. We have used, per litre final medium, 0·25 g. L-cysteine hydrochloride and 0·25 g. sodium thioglycollate together with 0·5 g. agar, and methylene blue or resazurin as an oxidation-reduction indicator. On this medium good growth was obtained of thirteen species of clostridia including strains of *Cl. tetani* and *Cl. oedematiens*. By adding the same supplements to half strength CYLG medium, a good storage medium was produced suitable for maintaining not only clostridia but also certain 'difficult' organisms such as streptococci and pneumococci.

Other media

One of the main features of the medium which we wish to emphasize is its versatility. We have given examples of various routine media which can be prepared from the CYLG base. With the exception of those media whose utility depends on an appreciable pH change it would appear to form an adequate base for most routine media.

SUMMARY

An easily prepared and readily reproducible culture medium for general use is described. A wide range of standard media can be prepared from it by simple modifications.

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REFERENCES

ALEXANDER, H. E. (1958). The Hemophilus group in *Bacterial and Mycotic Infections of Man*, ed. by R. J. Dubos, 3rd ed., p. 470. Philadelphia, Penn.: J. B. Lippincott.

BLOCK, R. J. & WEISS, K. W. (1956). *Amino Acid Handbook*. Springfield, Ill.: Charles C. Thomas.

BURNETT, G. W., PELCZAR, M. J. & CONN, H. J. (1957). Preparation of media in *Manual of Microbiological Methods*, by Society of American Bacteriologists' Committee on Bacteriological Technique, p. 37. New York: McGraw-Hill.

EULER, H. VON & SCHLENK, F. (1937). Co-zymase. *Hoppe-Seyl. Z.* **246**, 64.

FILDES, P. (1920). A new medium for the growth of *B. influenzae*. *Brit. J. exp. Path.* **1**, 129.

GLADSTONE, G. P. & FILDES, P. (1940). A simple culture medium for general use without meat extract or peptone. *Brit. J. exp. Path.* **21**, 161.

HIRSCH, A. & GRINSTED, E. (1954). Methods for the growth and enumeration of anaerobic spore-formers from cheese with observations on the effect of nisin. *J. Dairy Res.* **21**, 101.

LEIFSON, E. (1943). Preparation and properties of bacteriological peptones. I. Enzymatic hydrolysates of casein. *Bull. Johns Hopkins Hosp.* **72**, 179.

SCHLENK, F. (1951). Codehydrogenases I and II and Apoenzymes in *The Enzymes*, ed. by J. B. Sumner & K. Myrbäck, vol. 2, part 1, p. 250. New York: Academic Press.

SOCIETY FOR GENERAL MICROBIOLOGY. (1956). *Constituents of Bacteriological Culture Media*. Special Report ed. by G. Sykes. Cambridge University Press.