PUTRESCINE AND RELATED AMINES AS GROWTH FACTORS FOR A MAMMALIAN CELL LINE *

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Received October 25, 1963

Clonal growth of strain CHD-3 Chinese hamster cells in a synthetic nutrient mixture supplemented with small amounts of two purified protein fractions, fetuin and serum albumin, has been previously reported (Ham, 1963a, b). In order to avoid complications due to possible common factors carried by both proteins, initial studies of the requirement for each protein were carried out in the presence of the other. The ability of linoleic acid to replace serum albumin in the presence of fetuin has already been described (Ham, 1963b). In the current report, replacement of fetuin in the presence of albumin by a group of amines which stimulate growth of a number of microorganisms (Tabor et al. 1961) is described.

The test system is essentially as previously described (Ham, 1963b). The basal medium is nutrient mixture F10 (Ham, 1963a), modified by increasing the $CaCl_2$ level to 1.5×10^{-3} M., and the $MgSO_4$ to 1.0×10^{-3} M. (Ham, 1963b). The extra calcium and magnesium must be added immediately before use of the medium. If they are added in advance and the alkaline medium is allowed to stand without equilibration with 5% CO_2, a precipitate gradually forms and the ability of the medium to support growth is destroyed. Folinic acid, 1.0×10^{-6} M., has also been incorporated into the medium (Ham, 1962).

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* Contribution No. 210. Presented in preliminary form at the 145th meeting of the American Chemical Society, New York City, September 13, 1963.

The basal medium is placed in plastic tissue culture petri dishes (Falcon No. 3002), and fetuin or fetuin substitutes are added to it. The cell inoculum is diluted with basal medium containing 1000 μg/ml of serum albumin to a concentration of 2000 cells per ml. 0.1 ml of the albumin-containing cell suspension is then added to 5.0 ml of basal medium plus additives in each petri dish to yield a final concentration of 200 cells per dish and 20 μg of serum albumin per ml. When linoleic acid is used in place of serum albumin, it is added to the basal medium in the petri dishes at a concentration of 2.5×10^{-7} M., and the cell suspension is diluted in the protein free basal medium. Normal incubation time is 10 - 12 days.

The data and conclusions presented in this report are based entirely on short term clonal growth experiments with strain CHD-3 Chinese hamster cells which have been previously cultured in medium F10HIFC (Ham, 1963a). Further testing is necessary before the results can be applied to other cell lines or to long term growth of strain CHD-3.

In the absence of fetuin, attachment and stretching of trypsinized strain CHD-3 cells on the surface of the plastic petri dishes is essentially the same as that with fetuin.[1] Early division also appears to be normal, but by the fourth day the cells without fetuin begin to lag behind the controls, and by the tenth day they form only small abortive colonies, while the controls develop into much larger colonies estimated to contain 500 - 1000 cells. A minimum of about 5 to 10 μg/ml of fetuin is needed for satisfactory colony formation.

Growth of the fetuin-free controls tends to vary considerably, apparently depending on how much fetuin or fetuin-replacing activity is initially carried within the cells. Such background growth can be suppressed, thereby enhancing the observed response to fetuin and

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1. In the absence of fetuin, mammalian cells in general appear to attach and stretch more readily on tissue culture plastic surfaces than on glass surfaces.

fetuin substitutes, by depleting the stock cultures in basal medium without fetuin for 24 hours before the actual plating experiment. This procedure was utilized in gathering the data presented in this paper.

A variety of natural products considered unlikely to contain intact fetuin were tested for their ability to replace fetuin in promoting cell growth. A number of these including ultrafiltrates of chick and beef embryo extracts, and hydrolysates of yeast, blood, lactalbumin, and liver were found to be active. "Panmede," an ox liver digest prepared by Paines and Byrne, Ltd., Greenford, England, was found to be particularly active, and was selected as a starting material for partial purification of the activity.

Preliminary studies with ion exchange resins revealed that the activity was taken up by H^+ charged Dowex 50, and that it could be eluted with 1.0 N. NaOH. A large portion of the activity was not taken up by OH^- charged Dowex 1. Since these properties suggested an amine which contained no acidic groups, an attempt was made to volatilize the activity by bubbling a stream of air through a strongly alkaline solution of Panmede and into an acidic trap. Fetuin replacing activity was recovered in the acid trap.

At this point, a number of amines were tested for activity. Putrescine (1,4-butanediamine), spermidine (N-(3-aminopropyl)-1, 4-butanediamine), and spermine (N,N'-bis(3-aminopropyl)-1,4-butanediamine), all of which are known to be growth factors for Hemophilus parainfluenzae (Herbst and Snell, 1949) and a variety of other microorganisms (for review see Tabor et al., 1961), were found to be highly active in promoting growth in the absence of fetuin (Table 1). In the presence of albumin, these amines appear to replace completely the growth promoting ability of fetuin. On a weight basis, putrescine is at least 200 times as active as fetuin, since 2.0×10^{-7} molar putrescine (ca. 0.02 μg/ml) has a biological activity which is roughly equivalent to

that of 5 μg/ml of fetuin. On a molar basis, the three amines are equally active (Fig. 1).

TABLE 1

GROWTH RESPONSE OF STRAIN CHD-3 CELLS TO FETUIN AND FETUIN REPLACEMENTS

Additive*		Plating Efficiency** (per cent)	Colony Size*** (photocell units)
None		34	4
Fetuin	10 μg/ml	42	17
Panmede	10 μg/ml	50	13
Putrescine	1.0×10^{-6} M.	46	15
Spermidine	1.0×10^{-6} M.	51	14
Spermine	1.0×10^{-6} M.	51	18

* Basal medium: F10 with $CaCl_2$ 1.5×10^{-3} M. and $MgSO_4$ 1.0×10^{-3} M. plus folinic acid 1.0×10^{-6} M. and serum albumin (Albumisol, Merck, Sharpe, and Dohme) 20 μg/ml. ** All colonies visible to the unaided eye were counted, including small abortive colonies of less than 50 cells. *** Photometric determination of relative colony areas. For details see Ham (1963a).

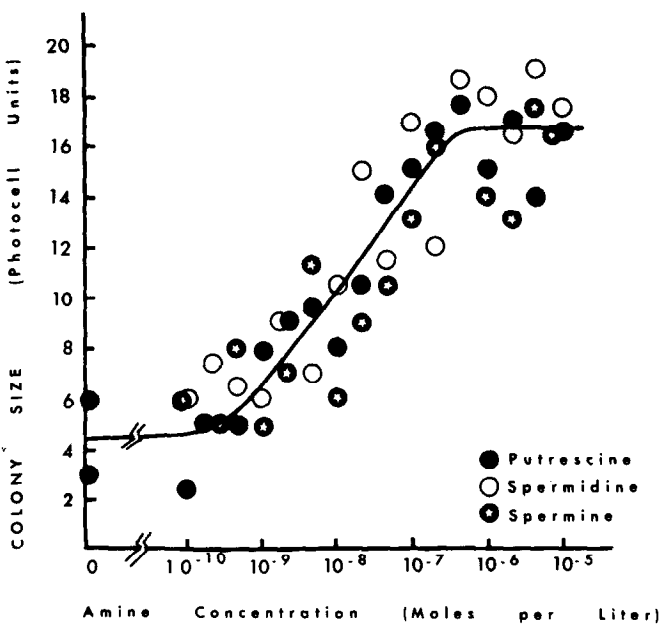

Fig. 1. Effect of putrescine, spermidine, and spermine on growth of strain CHD-3 cells. Basal medium: same as in Table 1. Incubation time: 12 days. Colony size is determined photometrically as previously described (Ham, 1963a).

The three amines also promote growth in the absence of serum albumin. Completely protein free clonal growth of strain CHD-3 has been obtained in media containing linoleic acid and any one of the three active amines. Under such conditions, growth of the surviving colonies has been good, but the plating efficiencies have been low (generally below 20 per cent). Preliminary studies indicate that the optimum concentration ranges of many of the components of nutrient mixture F10 are much narrower for completely protein free clonal growth than they are when protein is present. Thus, extensive titrations of nutrient concentrations will be necessary for the development of an optimally balanced medium for protein free clonal growth of strain CHD-3.

Turner et al. (1963) have reported that spermine is involved in thymidine and folinic acid metabolism in several strains of bacteria. Such mechanisms do not appear to be involved in the amine requirement of strain CHD-3, however, since the basal medium already contains both of these compounds.

Acknowledgements

I am grateful to Mrs. Jane Treber for excellent technical assistance and to Dr. Theodore T. Puck for his interest and suggestions. This work was supported by a grant from The National Foundation and a USPHS Training Grant to the Department of Biophysics, 5 T1 GM-781-05.

References

Ham, R. G., Exptl. Cell Research 28, 489 (1962).
Ham, R. G., Exptl. Cell Research 29, 515 (1963a).
Ham, R. G., Science 140, 802 (1963b).
Herbst, E. J. and Snell, E. E., J. Biol. Chem. 181, 47 (1949).
Tabor, H., Tabor, C. W. and Rosenthal, S. M., Ann. Rev. Biochem. 30, 579 (1961).
Turner, R. B., Lansford, E. M., Jr., Ravel, J. M. and Shive, W., Biochemistry 2, 163 (1963).