ma globulin fraction. For several reasons this material has not been considered as an antibody(8).

It should be noted that the present method dilutes the gamma globulin 4-10 fold. If this dilution should be undesirable, the gamma-globulin eluate may be applied directly to a carboxymethylcellulose (CM) column, equilibrated with pH 6.3, 0.0175 M PO_4 buffer onto which the gamma gobulin will be adsorbed(2, 3). It may be then eluted in a more concentrated form by 2 M NaCl in 0.05 M Na-phosphate pH 6.9. Any other concentration method may, of course, be used.

Summary. A relatively simple method for separation of gamma globulin from serum has been presented. The procedure has been applied to a number of antisera, and has been shown with them at least to give reasonably good localization of antibody in the gamma globulin fraction.

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Effect of Pluronic F68 on Growth of Fibroblasts in Suspension on Rotary Shaker. (25477)

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Recent investigations have established the feasibility of propagating certain strains of mammalian cells in suspension, and a number of technics have been described. These include the tumble tube of Owens *et al.*(1), the rotary shaker of Earle *et al.*(2), the roller tube of Graham and Siminovitch(3), the suspended stirrer (spinner culture) of Cherry and Hull(4), the fermentor of McLimans *et al.*(5), and a variety of modifications of the foregoing(6,7,8,9,10). Use of conventional rotary shakers to provide agitation eliminates the need for special equipment and the method is particularly suitable for experiments involving many cultures of relatively large volume. Formation of a non-cellular precipitate(11) under certain conditions, however, is a major drawback of the shaker method. In media containing serum, the precipitate is presumably protein in nature(11,12) and its formation is related to strain of cells and environmental conditions employed(11). Precipitation of constituents of the medium not only results in erratic growth of cells but imposes severe limitations on their use in studies of metabolism. This report describes the use of Pluronic F68 in shake cultures as a means of essentially eliminating the precipitate formed in a medium containing horse serum.

Materials and methods. The Pluronics† are blockpolymer surfactants prepared by adding ethylene oxide to both ends of a polyoxypropylene polymer. Pluronic F68, hereafter referred to as F68, is a solid with a molecular weight of approximately 8750. About 80% of the molecule, by weight, consists of hydophilic polyoxyethylene groups and the remainder of hydrophobic polyoxypropylene groups. Stock cultures of strain U12-29 fi-

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broblasts(13) were propagated continuously in Bellco-type spinner flasks‡ in medium 29 composed of S103(14) fortified with 20% (v/v) horse serum (NHS). The flasks were stoppered and contained ½ to ⅔ their volume of medium. Cells were maintained in pseudo exponential growth by replacing a portion of the suspension with fresh medium at appropriate intervals. Aqueous solutions containing 5 to 25% F68 were sterilized by filtration through Selas§ candles of 02 porosity. All glassware in both spinner and shake cultures was siliconized with saturated solution of silicone‖ in carbon tetrachloride. After draining, the flasks were heated at 160°C for 45 minutes. Experiments with shake cultures were performed as follows: Aliquots of cell suspension from spinner cultures were centrifuged and the supernatant discarded. The cells were suspended in experimental medium at concentration of 0.3 to 0.6 × 10^5 cells/ml and 75 ml was added to each of a series of 125-ml Florence flasks. The flasks were gassed (45% O_2, 5% CO_2 and 45% N_2), stoppered, and incubated at 37°C on a rotary shaker¶ at 200 rpm. Samples were removed at 24 to 72 hour intervals and the cells enumerated by the citric acid procedure employed in this laboratory(15).

Results. When medium 29 was shaken at 37°C in absence of cells, precipitation was evident within a few hours and was usually complete in 24 to 48 hours as judged by volume of pellet obtained upon centrifugation of aliquots of the medium at 1000 × G for 15 minutes. Media prepared with 10 different lots of serum all yielded considerable precipitate. The quantity and general appearance of the precipitate varied with the lot of serum employed. Some media became opalescent and considerable amorphous debris accumulated, whereas in others a granular precipitate was formed. When medium 29 was supplemented with 0.05 to 5% F68, precipitation of components of the medium was virtually eliminated. At these concentrations of F68 most sera remained clear for the experimental period of 7 to 10 days. In a few instances a small amount of granular-type precipitate was evident after 2 to 3 days, but this did not increase appreciably on continued shaking. With media prepared from a limited number of sera, F68 was effective in either preventing or markedly reducing precipitation in concentrations as low as 0.01%. The results of preliminary experiments indicate that F68 is also effective in reducing precipitation in similar media prepared from human serum.

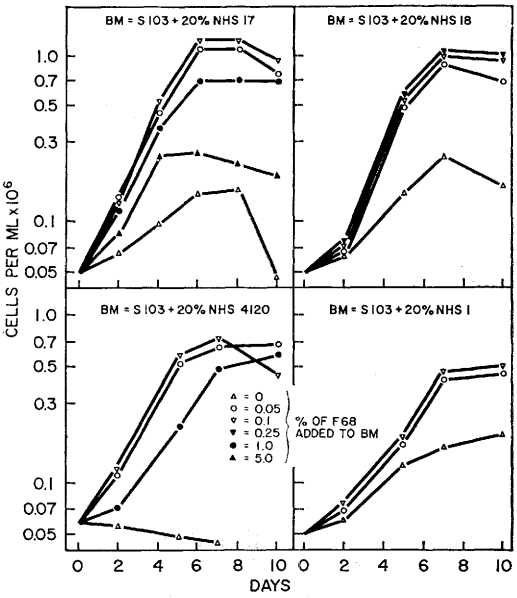

FIG. 1. Growth of U12-29 fibroblasts in shake cultures as a function of concentration of Pluronic F68 in medium 29. Each point on curves represents avg values for 2 or more experiments. BM refers to basal medium to which F68 and horse serum was added.

The effect on proliferation of U12-29 fibroblasts in shake cultures of adding F68 to basal medium is illustrated in Fig. 1. Amount of growth in absence of F68 is consistently less than one half that obtained when the Pluronic is added to the medium. The results varied with the lot of serum employed. For example, some growth was obtained in absence of F68 in media prepared with either NHS 17, 18 or 1, whereas the cells began to degenerate in a few days in a medium con-

‡ Flasks manufactured by Bellco Glass, Vineland, N. J.

§ Porcelain filters manufactured by Selas Corp., Philadelphia, Pa.

‖ A silicone lubricant manufactured by Dow Corning Corp., Midland, Mich.

¶ Model S-3 shaker manufactured by Brunswick Scientific Co., New Brunswick, N. J.

taining NHS 4120. Likewise, significantly less growth was obtained in presence of F68 with NHS 1 than with the other 3 sera. Differences of the same order of magnitude have been observed among additional sera not shown in Fig. 1. Variations in ability of different sera to promote growth are not unique to the shaker system since individual lots exhibit the same relative activity in stationary and spinner cultures.

A subline of strain U12-29, designated U12-79, grows well in stationary flasks in a medium composed of S103 fortified with 5% dialysed horse serum, but fails to proliferate in either shake or spinner cultures. Bryant *et al.*(16) observed that addition of the lower viscosity grades of the substituted cellulose polymer, Methocel** permits growth of the NCTC 2071 strain of fibroblasts on the shaker in a chemically defined medium. In the absence of Methocel the cells degenerate when shaken but proliferate in the same medium in stationary flasks. The results obtained with U12-79 indicate that F68 does not function as does Methocel in facilitating growth of cells in minimal media in shake cultures. The growth enhancing activity of F68 in shake cultures appears to be directly related to its ability to prevent precipitation of protein from the medium. Thus, growth curves of U12-29 in shake cultures containing low levels of F68 (Fig. 1) are indistinguishable from those obtained in a Pluronic-free medium in spinner flasks where precipitation is minimal.

Summary. A medium composed of the defined solution S103 supplemented with 20% horse serum yields a noncellular precipitate when agitated on a rotary shaker. This precipitation results in loss of from 25 to 100% of growth-promoting activity of the medium for strain U12-29 fibroblasts in shake cultures. Addition of the polyglycol, Pluronic F68, to the medium largely eliminates precipitation. Under these conditions, growth of U12-29 in suspension on a rotary shaker is comparable to that obtained in spinner cultures.

** Manufactured by Dow Chemical Co., Midland, Mich.

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