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Establishment of Permanent Cell Strains from Human Adult Peripheral Blood

Cultures of cells from the buffy coat of the blood were described by Carrel and Ebeling in 1922[1], and since then they have been used by many workers. Several have described the appearance of fibrocytic cells in these cultures[2]. They have been reported as early as one day after explantation, and Hulliger[2], using mainly rabbit buffy coat, found that within one to three weeks cultures of fibrocytic cells were frequently formed in which a high level of mitosis occurred. It would be useful to be able to establish strains of cells from individuals whenever they are required for certain genetic studies, and since the peripheral blood is the easiest tissue to obtain aseptically from volunteers, the possibility that permanent strains might be developed from this source was investigated. Strains were established from four out of five bloods. The technique used is as follows.

15–20 ml. of blood are collected in sterile siliconed tubes and buffy coat cultures are prepared in 50 ml. conical flasks, essentially by the method described by Allgöwer[2]. After one week a fluid medium consisting of 20 per cent horse or calf serum in Eagle's medium[3] (with phenol red) is added to the plasma clot cultures. About this time the cultures enter a static phase in which they remain for a period which varies from a few days to many weeks. The only evidence of the presence of surviving cells is in some cases the progressive fall in the *p*H of the medium. This is observed daily, and when it falls to 7·0 the fluid phase is renewed. Quite suddenly rapidly growing colonies of cells appear. These can be trypsinized in the usual manner and established in continuous cultivation. They resemble other 'fibroblastic' strains in morphology and grow very well in 20 per cent calf serum in Eagle's medium. They can be cloned quite easily by the dilution technique[4]. The chromosomes have been identified as human by Prof. Pontecorvo and Mr. C. G. Elliott of the Genetics Department of this University.

The origin of these cells is by no means certain. The following suggestions have been made: (1) They represent transformed monocytes or lymphocytes; (2) they are cells carried into the vein during venepuncture; (3) they are tissue fibroblasts or other cells, not usually listed among the leucocytes, which exist in small numbers in the peripheral blood. In view of the regularity with which they can be obtained, I feel that the second explanation is unlikely. But since their origin is at present uncertain they are best described as normal adult human buffy coat cells. We have four strains, HERT 1, HERT 2, HERT 3 and HERT 4, isolated from four different individuals and maintained in continuous culture for periods varying from six months to a year, with a minimum of thirty serial passages.

If these are normal blood elements, no matter what their precise origin, it seems that Allgöwer's suggestion that they give rise to the fibroblasts of granulation tissue has some justification. Also it seems not unlikely that many of the 'fibroblastic' strains of cells isolated from a variety of tissues may, in fact, have their origin from these blood cells. Finally, in view of the readiness with which the colonies grow from individuals the danger of using unfiltered serum in culture media is obvious.

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Protoplasts of Group *A* Beta-hæmolytic Streptococci

This communication records the preparation from strains of group *A* streptococci of bodies which appear to conform to the rigid definition of 'protoplasts' recently offered by Brenner *et al.*[1]. They have been prepared by enzymic digestion of living cells.

There are two enzyme systems which attack isolated cell walls of group *A* streptococci: the *Streptomyces albus* mixture[2] and the enzyme produced during bacteriophage lysis of group *C* streptococci[3]. Both enzyme systems have been used successfully to prepare protoplasts, but the latter enzyme system is the more suitable since the streptomyces mixture contains an enzyme(s) which attacks the protoplast membrane.

Our general method has been to incubate the streptococcus (Lancefield group *A*, type 14 concentration 10^8 orgs./ml.) with the enzyme in 2 *M* sodium chloride or 2 *M* sucrose for 30 min. at 37° C. Little change in turbidity, as measured spectrophotometrically, occurs in this time and microscopical examination of air-dried stained smears of the suspension shows discrete spherical particles. Under dark-ground illumination they are indistinguishable from the original streptococcus (except possibly smaller). The suspension is centrifuged, the precipitate washed twice with 2 *M* salt solution and resuspended in a medium of suitable composition for further studies. The properties of these protoplasts, are as follows:

Osmotic sensitivity. Suspension of the protoplasts in distilled water or dilute (less than *M*) buffer brings about immediate lysis with the release of internal components of the organism. Under appropriate