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Mucopolysaccharide Production By Fibroblasts Derived from Human Buffy-Coat Cultures *in vitro*.* (29328)

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Grossfeld(1) has shown that fibroblasts from different tissues cultivated *in vitro* are able to produce acid mucopolysaccharides (AMPS) and release them into the medium. AMPS belong to the group of hyaluronic acid and chondroitin sulphates A and C, are highly polymerized, and give positive mucin clot and hyaluronidase tests(2).

Since fibroblast-like cells are known to originate in cultures of buffy-coat and hemapoietic tissues(3,4), it seems of interest to study the AMPS production in human white blood cell cultures undergoing fibroblastic transformation.

Materials and methods. Peripheral human blood buffy-coat was obtained by venipuncture and centrifugation at 600 × *g* with 0.1 mg/ml of heparin (Liquemine Roche) for each 5 ml of blood and phytoagglutinine (Difco), 0.25 ml for each 10 ml of blood. The first 1 or 2 ml extracted from the vein was discarded to avoid contamination with tissue cells. Buffy-coats from 10 ml of blood were explanted in Earle's flasks with a layer of isologous plasma and 5 ml of liquid medium containing yeast extract-Eagle medium-lactalbumin hydrolysate-peptone(5) and 5% chicken embryo extract.

Turbidimetric tests of hyaluronidase according to Seastone and Kass(6) were performed as previously described(7) in samples of culture media taken every 4 days. The medium was centrifuged to eliminate cells and debris that might interfere with the turbidimetric tests. Incubated medium without explants was used as a blank. Determinations were also made on aliquots of homogenized cells before and after cultivation. The cells were homogenized in phosphate buffer pH 6.5 (0.1 M) with Potter-Elvehjem homogenizer and centrifuged at 800 × *g*. The supernatant was taken for analysis.

Hyaluronidase from Benger Lab. Lt. was used (Hyalase Benger). It was found that 500 U Benger was the optimum necessary to reduce turbidity to 50%. Standard curves were made with pure hyaluronic acid prepared at Prof. Karl Meyer's Laboratory.

Results. a) *Appearance of fibroblast-like cells.* Fibroblast-like cells start to appear about 5 days after explantation (Fig. 1). Some of the cells have the characteristic appearance of macrophages and others have distinct fibroblast features. By the second week of cultivation, a thick layer of fibroblasts can be observed. b) *Release of AMPS in the medium.* Estimation of AMPS in the first change of medium (4 days) shows a high content, amounting to about 50% of the total AMPS of the explanted cells. This may be due to release of AMPS from degenerating granulocytes, platelets and cell debris which are found in suspension in the liquid medium. Thereafter, release of AMPS into the medium

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TABLE I. Net Production of Acid (μg) Mucopolysaccharides During 30 Day Period.

Total explanted content (A) (cells + plasma)	Release in culture medium (B)*	Harvested cells (C)	Net amps production (B + C − A)
793 ± 67†	1,475 ± 270†	414 ± 86†	1,100 ± 175†

* After deducting the medium blank.
† Mean and standard error from 6 different experiments. Each determination was made in duplicate.

decreases, concurrently with the decrease in cell destruction, until the second week of cultivation. At this stage no cell destruction can be detected, fibroblastoid transformation becomes widespread and the AMPS titre in the medium increases gradually till the third or fourth week (Fig. 2). Then, free AMPS decreases again, perhaps because the cultures are no longer growing or because AMPS are incorporated in a more stable and less soluble structure. The cultures showing a high ratio of growth and transformation showed high AMPS values. The presence of fresh chick embryo extract is necessary for AMPS production, as has been also pointed out for other tissues by Grossfeld(2). c) *AMPS content of blood cells and net production in cultures.* In 6 experiments the net production of AMPS was determined by calculating the difference between total amount in the explant plus original content in the plasma and fluid medium, and total AMPS harvested in the medium and the cells throughout the period of cultivation. Net production of AMPS in terms of hyaluronic acid was approximately 1 mg per flask (Table I) for the 30 day period of observation.

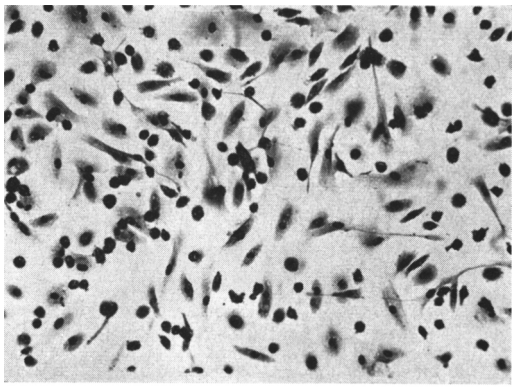

FIG. 1. Onset of fibroblast transformation in human buffy-coat culture. One week of cultivation—Giemsa stain (318 ×).

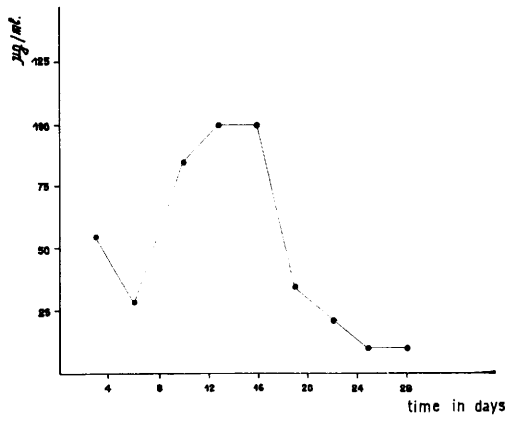

FIG. 2. Release of acid mucopolysaccharides in culture medium.

Discussion. High values of AMPS obtained during the first washing of liquid medium can be explained by the destruction and elimination of granulocytes and platelets. Both kinds of cells contain AMPS(8,9).

Fibroblast-like cell transformation takes place in cultures of blood and hemapoietic organs and appears to be confined to mononucleated cells. Studies on the capacity of these cells to make hydroxyproline(10) and collagen(11) reveal their mesodermic potentalities. Their morphological appearance is apparently related to cultural conditions still not well defined(12). Cells from mesodermal origin taken from different sources tend to acquire *in vitro* a fibroblastic shape and synthesize products characteristic of connective tissue matrices such as AMPS and collagen. It would appear, therefore, that these relatively undifferentiated mesenchymal cells are able to differentiate morphologically and functionally towards the fibroblastic type when culitvated on a solid substrate under suitable conditions.

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Specific Immunoprecipitation of Mouse Thyrotropins in Plasma and Thyrotropic Tumor Extracts.* (29329)

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Attempted immunoassay of thyrotropic hormone (TtH) of mice, begun by us many years ago, was unsuccessful until 2.3 mg of a highly purified TtH, free from detectable serum protein, was obtained from R. W. Bates (Nat. Inst. Arth. Met. Disease). An antiserum (a-mouse TtH) produced with this purified TtH gave specific precipitation with as small a quantity as 0.002 ml of serum of highly functional thyrotropic tumor (TtT)-bearing mice. This is estimated to contain somewhat less than 1 mu of TtH. This a-mouse TtH gave no reaction with 0.2 ml serum specimens of mice bearing adrenotropic (AtT) and mammo-somatotropic (MStT) tumors and with normal sera. In immunoelectrophoresis of the serum, the TtH was localized in the globulin region.

Materials and methods. Preparation of a-mouse TtH. The mouse TtH of Bates contained 12.5 u/mg. It was prepared by successive percolation and chromatographic fractionation(1,2). Two units, incorporated in complete Freund adjuvant (Difco), were injected into each of 2 rabbits intramuscularly, and 4 weeks later, 3 units in saline were injected intraperitoneally; 2 weeks later, a third injection of 2 units was given subcutaneously. The rabbits were exsanguinated 12 days after the last injection. Serum 120, which gave a strong and specific reaction with TtH and was used in the present study, will be referred to as "a-mouse TtH." The second antiserum was poor.

Antiserum against bovine TtH (Thytropar, Armour), to be referred to as "a-beef TtH," was produced in the same manner. Each rabbit was given 14 units of Thytropar during a period of 3 months. Antisera prepared against plasma of hypophysectomized mice (a-mouse serum) were prepared in the same manner.

TtT. In mice, TtT was induced by radiothyroidectomy with I^{131}(3) and was carried in successive passages in isologous LAF_1 mice. A functional, transplantable thyrotropic pituitary tumor was induced in rats by Astatine^{211} (Sinha, Kunii and Furth, to be published). Bioassays for TtH were made with McKenzie's assay(4).

Agar-gel precipitation (Ouchterlony) test. The plates were prepared with Noble agar (Difco), phosphate buffer, and merthiolate by standard procedure. Petri dishes with wells

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