Vol. 81, No. 1, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 15, 1978 Pages 254-259

STEROL SYNTHESIS IN GRANULOCYTES

Stephen R. Loveless and Howard R. Slater

Department of Genetics, University of Aberdeen, Scotland.

Received January 16,1978

Summary A method for separating granulocytes from human blood is described. These cell preparations are shown to synthesise digitonin-precipitable sterols, but at lower rates than mononuclear cells separated from the same blood sample. Delipidated serum is shown to stimulate granulocyte synthesis and low density lipoproteins depress it. Cholesterol and squalene have been detected amongst the lipids synthesised by granulocytes using Thin Layer Chromatography.

Conflicting reports have been made on the ability of granulocytes to synthesise sterols. One study on rat cells (6) has reported a total absence of synthesis whereas another describes active cholesterogenesis (7). A study of human granulocytes (5) describes an ability to synthesise squalene but not sterols. We report a method of isolating human granulocytes which demonstrate both squalene and sterol synthesis and describe some factors which influence it.

METHODS

Cells were separated from 20ml of heparinised venous blood collected after a 12-14 hour fast. The blood was mixed in a siliconised tube with half its volume of Plasmagel (Laboratoire Roger Bellon, Neuilly, France) and allowed to stand at room temperature for 45 minutes. The upper leukocyte-rich fraction was removed and layered onto Lymphoprep (8). After centrifugation at 400g for 25 minutes, mononuclear cells form a band at the Plasmagel-Lymphoprep interface; granulocytes and residual red blood cells are sedimented to the bottom of the tube. Cells were washed with calcium-free Krebs Ringer Phosphate Buffer pH 7.4 and aliquots counted using an Improved Neubauer counting chamber. Slides for differential counting were prepared using a cytocentrifuge (Shandon Elliot) and Wright's stain.

Cells (2×10^6) were suspended in RPMI 1640 media (Gibco Biocult Labs Ltd.) containing 100 U of penicillin/ml and 100 μg of streptomycin/ml with 1.5ml delipidated serum (1mg/ml protein), 0-0.5ml low density lipoprotein (72mg cholesterol/dl) and 20 μl [U-^{14}C] acetate (22.4 Ci/mol). All volumes were made up to 3.52 ml with RPMI 1640. After equilibrating the media with 95% O_2/5% CO_2, the cells were incubated at 37°C for 0-20 hours.

0006-291X/78/0811-0254$01.00/0

Human serum was delipidated by the method of Cham and Knowles (9). Human low density lipoprotein was separated by sequential flotation in the ultracentrifuge (10) and stabilised in a 4% solution of bovine serum albumin. All sera or serum fractions were extensively dialysed against buffered saline and sterilised by membrane filtration.

Incubations were terminated by the addition of 1ml 12 N NaOH and 4.5ml methanol to each culture. The mixtures were saponified for one hour at 70°C and neutral lipids extracted into petroleum ether b.p. 60-80°. β -hydroxy sterols were precipitated from the petroleum ether with digitonin (11) and washed twice with 80% ethanol and once with ether. The digitonides were dissolved in 0.5ml methanol, added to scintillation fluid and radioactivity measured.

Petroleum ether extractions were chromatographed in benzene on thin layer silica gel plates with cholesterol and squalene standards. Radioactive spots were photographed in a spark chamber (Birchover Instruments) and scraped into scintillation fluid for radioactive counting.

RESULTS

The granulocyte preparations incorporate [^{14}C] acetate into digitonin-precipitable sterols as shown in fig.1. The cell preparation method used yielded white cell cultures containing at least 95% polymorphonuclear neutrophils. Red blood cell contamination varied depending on the efficiency of the Plasmagel separation stage but remained below 20 red blood cells per leukocyte. To maximise the yield of viable granulocytes, no attempt was made to remove these residual blood cells. Appropriate controls were included to correct for possible use of [^{14}C] acetate by red blood cells but no competition for substrate or sterol synthesis was detectable in these cells.

As with mixed leukocytes (12), sterol synthesis in granulocytes was stimulated by delipidated serum as shown in fig.2.

To identify radioactive products, petroleum ether extractions of saponified granulocyte cultures were resolved by thin layer chromatography as shown in fig.3. Four radioactive spots were repeatedly observed, two of which co-chromatographed with cholesterol and squalene standards.

The rate of sterol synthesis of granulocytes has been compared with that of mononuclear cells separated from the same blood sample as shown

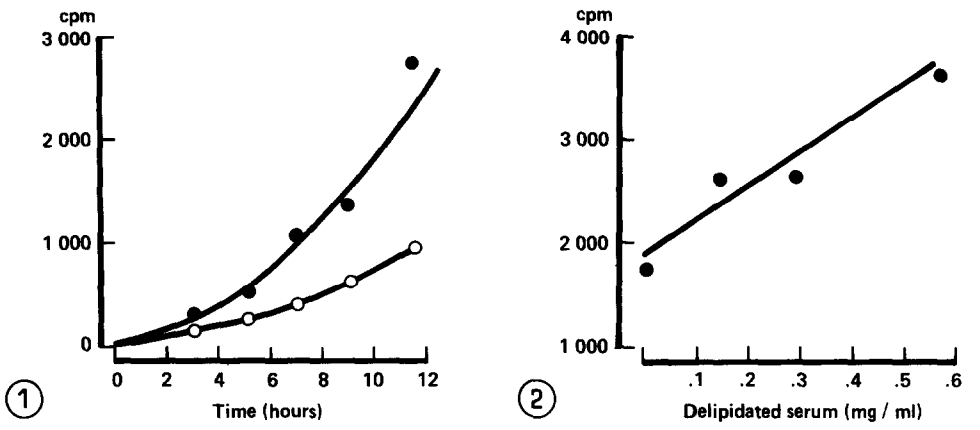


FIGURE 1 Time Course of Incorporation of ^{14}C Acetate into Digitonin-Precipitable Sterols by Granulocytes (○) and Mononuclear Leucocytes (●).

Granulocytes and mononuclear cells were separated from 20mls of venous blood collected from a healthy female donor aged 45 years as described in "Methods". Flasks containing 2 x 10^6 granulocytes or mononuclear cells were incubated with 4 μCi of [^{14}C] acetate for 3-11.5 hours; the concentration of delipidated serum was 426 μg protein/ml. Digitonin-precipitable sterols were extracted and radioactivity measured.

FIGURE 2 The Effect of Delipidated Serum on Synthesis of Digitonin-Precipitable Sterols by Granulocytes.

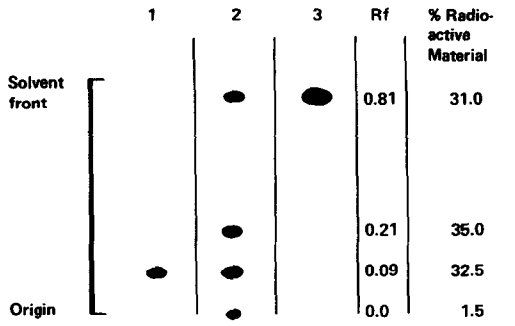
Granulocytes were separated from 20mls of venous blood collected from a healthy female donor aged 45 years as described in "Methods". Flasks containing 2 x 10^6 granulocytes each were incubated for 20 hours with 4 μCi of [^{14}C] acetate in variable concentrations of delipidated serum (0-572 μg protein/ml); digitonin-precipitable sterols were extracted and radioactivity measured.

in fig.1. In the latter stages of incubation, where incorporation rates approach linearity, mononuclear cells synthesise at approximately four times the rate of granulocytes.

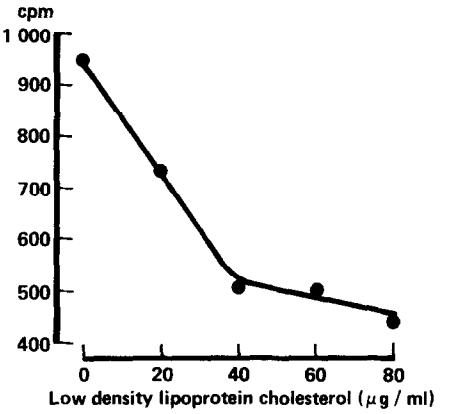
Purified low density lipoprotein has been shown to be a potent inhibitor of sterol synthesis in human mononuclear leukocytes (4). Such inhibition has been demonstrated in granulocytes as shown in fig.4.

DISCUSSION

The stimulation of granulocyte sterol synthesis follows a time course very similar to that of mixed leukocytes (1) and mononuclear cells



(3)



(4)

FIGURE 3 Thin Layer Chromatography of Neutral Lipids Synthesised by Granulocytes.

Granulocytes were separated from 20ml of venous blood collected from a healthy female donor aged 32 years as described in "Methods". Three flasks, each containing 2 x 10^6 granulocytes, were incubated for 20 hours with 4 μCi of [^{14}C] acetate; the concentration of delipidated serum was 426 μg protein/ml. After saponification, neutral lipids from each flask were extracted into petroleum ether and pooled. The solvent was then evaporated under nitrogen and lipids redissolved in 50 μl of petroleum ether. 20 μl of this extract were chromatographed in benzene on a silica gel thin layer plate along with cholesterol and squalene standards.
Lane 1 - 20 μl cholesterol standard; 10mg/ml.
Lane 2 - 20 μl petroleum ether extract.
Lane 3 - 3 μl squalene standard.
Radioactive spots were located by photography in a spark-chamber and removed for measurement of radioactivity by scintillation counting.

FIGURE 4 The Effect of Low-Density Lipoprotein on Synthesis of Digitonin-Precipitable Sterols by Granulocytes.

Granulocytes were separated from 20ml of venous blood collected from a healthy female donor aged 32 years as described in "Methods". Flasks containing 2 x 10^6 granulocytes each were incubated for 20 hours with 4 μCi [^{14}C] acetate in variable concentrations of low-density lipoprotein (0-80 μg cholesterol/ml); the concentration of delipidated serum was 426 μg protein/ml. Digitonin-precipitable sterols were then extracted and radioactivity measured.

(fig.1). When incubated in a cholesterol-free, delipidated serum-containing medium, the cells begin to synthesise sterols after a short lag-period. It seems likely that induction of enzymes necessary for endogenous synthesis takes place during this stage. The rates of sterol synthesis observed

cannot be explained by the very small numbers of contaminating mononuclear cells.

Granulocytes, like other white blood cells, show a minimum of sterol synthesis in the absence of delipidated serum (fig.2). The nature of this stimulation is unknown although loss of sterols from the cells into the medium may be involved (1).

Sterol synthesis in granulocytes can be inhibited by low density lipoproteins (fig.3) as previously shown in skin fibroblasts (13), leukocytes (12) and human arterial smooth muscle cells (14). As with these other cell types, the effective concentration of low density lipoprotein required are much lower than those found in serum suggesting that endogenous sterol synthesis is normally repressed.

Granulocytes have been shown to synthesise less actively than mononuclear cells separated from the same blood sample. It thus seems advisable to avoid the use of mixed leukocyte cultures although these have proved useful in the definition of genetic abnormalities of lipid metabolism (1,2,3). For the purpose of genetic screening, granulocytes offer a more convenient material than cultured fibroblasts. Since lymphocytes are known to consist of a number of functionally defined subpopulations, granulocytes may offer an equally convenient and more homogenous cell type.

ACKNOWLEDGEMENTS

We thank Ms Valerie Murphy for excellent technical assistance and Mr A M Cumming for supplying purified human low density lipoprotein.

REFERENCES

1. Fogelman, A.M., Edmonds, J., Seager, J. and Popjak, G. (1975) J. Biol. Chem. 250/6, 2045-2055.

2. Higgins, M.J.P., Lecamwasam, D.S. and Galton, D.J. (1975) Lancet 2, 737-740.

3. Higgins, M.J.P. and Galton, D.J. (1977) Eur. J. Clin. Invest. 7, 309-313.

4. Ho, J.K., Brown, M.S., Bilheimer, D.W. and Goldstein, J.L. (1976) J. Clin. Invest. 58, 1465-1474.

5. Fogelman, A.M., Seager, J., Edwards, P.A., Hokom, M. and Popjak, G. (1977) Biochem. Biophys. Res. Comm. 76, 167-173.

6. Rodwell, V. and Young, N.L. (1977) J. Lipid Res. 18, 572-581.

7. Polsky, F. and Siperstein, M.D. (1973) Clin. Res. 21, 88.

8. Boyum, A. (1976) Scand. J. Immun. 5, suppl. 5.

9. Cham, W.E. and Knowles, B.R. (1976) Clin. Chem. 22/3, 305-309.

10. Hatch, F.T. and Lees, R.S. (1968) Adv. Lipid Res. 6, 1-68.

11. Sperry, M.W. (1963) J. Lipid Res. 4/2, 221-225.

12. Fogelman, A.M., Seager, J., Edwards, P.A. and Popjak, G. (1977) J. Biol. Chem. 252/2, 644-651.

13. Brown, M.S., Dana, S.E. and Goldstein, J.L. (1973) Proc. Nat. Acad. Sci. USA. 70/7, 2162-2166.

14. Bierman, E.L. and Albers, J. (1977) Biochim. Biophys. Acta 488, 152-160.