SHORT COMMUNICATION

DETECTION OF MUCOPOLYSACCHARIDOSES BY SULPHATE INCORPORATION INTO STIMULATED LYMPHOCYTES

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SUMMARY

1. A new technique has been developed for the detection of inborn errors of mucopolysaccharide metabolism by measurement of the incorporation of radioactive sulphate into lymphocytes stimulated by phytohaemagglutinin.

2. Incorporation of $^{35}\text{SO}_4$ was much higher in lymphocytes of patients with mucopolysaccharidosis than in healthy subjects.

3. This technique offers a rapid, simple, reliable screening procedure for the detection of mucopolysaccharidoses.

Key words: mucopolysaccharidoses, lymphocyte, radioactive sulphate incorporation.

Mucopolysaccharidoses are inherited disorders of mucopolysaccharide metabolism, in which mucopolysaccharides accumulate in various tissues of the body (Brante, 1952). The various forms of mucopolysaccharidosis can be divided into six groups on the basis of clinical, biochemical, morphological and genetical differences, and some of these can be further divided into subgroups (McKusick, 1969). Clinical diagnosis of these diseases can be supported by the detection of elevated mucopolysaccharide excretion in the urine (McKusick, 1966), or the metachromatic staining of leucocytes (Bowman, Mittwoch & Schneiderman, 1962; Foley, Danes & Bearn, 1969) and fibroblasts (Danes & Bearn, 1965); none of these methods is entirely reliable. An exact diagnosis is possible by use of the so-called ‘corrective factors’ (Kresse & Neufeld, 1972), but this method is very time-consuming and tedious. A simpler procedure was therefore sought for the detection of these diseases.

Matalon & Dorfman (1966) and others (Fratantoni, Hall & Neufeld, 1968) found that fibroblasts of patients with Hurler’s and Hunter’s syndromes and other mucopolysaccharidoses have an abnormal pattern of $^{35}\text{SO}_4$ incorporation into mucopolysaccharide.

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Since the leucocytes of patients with mucopolysaccharidoses also have mucopolysaccharide inclusion bodies like those found in the fibroblasts it might be expected that mucopolysaccharidoses could be detected by the analysis of $^{35}\text{SO}_4$ incorporation into leucocytes.

We have examined incorporation of $^{35}\text{SO}_4$ into lymphocytes of thirteen normal control children and seventeen children with the following categories of mucopolysaccharidosis (MPS): one Hurler (MPS I), two Hunter (MPS II), nine Sanfilippo (MPS III), four Morquio (MPS IV) and one Maroteaux–Lamy (MPS VI). In addition we examined three so-called 'variant' forms of mucopolysaccharidosis (McKusick, 1969) and one case of mucoviscidosis (Kraus, Antonowicz, Shah, Lazarus & Schwachman, 1971). The diagnoses were established on the basis of family pedigrees, clinical and radiological data and measurement of mucopolysaccharide content in 24 h urine samples (Calatroni, 1972).

METHODS

The method described below was developed on a patient with Hurler's syndrome. Leucocytes were isolated from 10 ml of heparinized venous blood (2-5 mg of heparin; 100 units/mg) by sedimentation, and were cultured at 37°C in Eagle's minimal essential medium supplemented with 20% calf serum and phytohaemagglutinin P (PHA; 0.05 ml/10 ml of medium). MgCl$_2$ was substituted for MgSO$_4$. The cells were incubated for 60 h and 70 $\mu$Ci of carrier-free Na$_2^{35}$SO$_4$ was then added. The samples always contained about 1 x $10^7$ cells/10 ml of incubation medium, assessed by counting the cells in a haemocytometer. At given intervals aliquots were taken and washed eight times with NaCl (0.154 mol/l). The washed cells were treated twice with 5 ml of boiling 80% ethanol for 1 min (Fratantoni et al., 1968). Radioactivity in the insoluble residue was measured on paper filters in a scintillator solution containing (per 1 of toluene) 2,5-diphenyloxazole, 6 g, 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene, 75 mg, in a Packard liquid-scintillation spectrometer. The viability of cells was established by incorporation of $[^3\text{H}]$thymidine into trichloroacetic acid-precipitable material.

RESULTS AND DISCUSSION

The conditions described above were found to give maximal differences between the incorporation patterns of the normal subject and the patient with Hunter's syndrome. Phytohaemagglutinin stimulation was essential since without PHA no difference was found in the radioactive sulphate incorporation pattern at 1, 2, 3, 4, 5 and 24 h. With TC 199 or Eagle's medium (with or without MgSO$_4$) with 20% calf serum, or 20% human AB serum or 10% calf + 10% foetal calf serum, the optimal effect was found with the combination described above. The extent of incorporation depends on the age of culture; the greatest differences were detected with 1–5 h labelling after 60 h incubation with PHA.

Under these conditions radioactive sulphate incorporation into mucopolysaccharides of control, mucoviscidoses and 'variant' lymphocytes was negligibly small in contrast to the patients with MPS (Fig. 1a). In the patient with Hurler's syndrome the test was carried out six times during a 1 year period, with similar results. Similar results were obtained with the two patients with Hunter's syndrome and on the patients with Morquio's syndrome. Problems were encountered, however, in the Sanfilippo's syndrome group. In one patient out of nine the incorporation was even higher than in the other mucopolysaccharidosis types. On the other
hand, four cases exhibited only moderately high incorporation, and in four cases the incorporation was as small as in the controls (Fig. 1b). These differences could reflect a heterogeneity of the Sanfilippo group (Kresse, Wiesmann, Cantz, Hall & Neufeld, 1971; Kresse & Neufeld, 1972; O’Brien, 1972; Dean, Muir & Benson, 1973).

On the basis of these results we believe that the observed pattern of radioactive sulphate incorporation is a consistent characteristic for several forms of mucopolysaccharidoses. The method is simpler and faster than those using fibroblast cultures, and should be useful in routine screening for mucopolysaccharidoses. The results of a similar study on heterozygotes will be published elsewhere.

REFERENCES


