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Tritium Labelling of Cells *in vitro*

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Alkyl alkanesulfonates tritiated in the alkyl groups can be utilized to introduce tritium into protein molecules. On this basis a rapid and simple method for isotope labelling of living cells has been developed. The labelling can be performed under conditions where no harmful effects on the cells accompany the treatment.

In a recent paper[1] we described a procedure for the tritium labelling of antibodies. This method is based on the introduction of tritiated ethyl groups into protein molecules by the use of ethyl methanesulfonate (EMS). At ordinary pH values, the ethyl groups become attached mainly to the free carboxyl groups of proteins, and it was shown that the label is stable to hydrolysis and that it does not influence the physico-chemical and immunological properties of a protein to any considerable degree.

EMS, which has a halflife of 10.4 h in water at 37°C reacts too slowly to be suitable for the labelling of living cells as the time during which these are exposed to the rather toxic and non-physiological environment of the labelling solution generally has to be kept as short as possible. The isopropyl homologue (*i*-PMS), however, has a corresponding halflife of only 13.6 min. Although its reaction with carboxyl groups, in relation to the hydrolysis, proceeds more slowly than that of EMS, a factor of at least five in reaction velocity is gained by using *i*-PMS instead of EMS according to the values of rate constants given by Ross.[2] Thus, a cell suspension can be effectively labelled within a reaction time of a few minutes. Table 1 demonstrates the degrees of labelling corresponding to the shortest possible reaction time (mixing at ice-temperature, centrifugation and separation) and to a complete reaction (about five halflives) at 37°C.

The sulfonic acid formed by hydrolysis makes it necessary to buffer the solution well. As the solution must be maintained close to isotonic, the maximum concentration of *i*-PMS which can be conveniently utilized is about 0.1 %. At the four times greater concentration used in the experiment above, some cells hemolysed during the incubation due to the pH drop.

Generally, alkyl alkanesulfonates have mutagenic as well as cytotoxic effects.[3,4] An experiment with lymphocytes was therefore performed to

Table 1. Tritium labelling of erythrocytes (10 % suspension in isotonic saline solution buffered to pH 8) by reaction with 0.4 % tritiated *i*-PMS.

Reaction time, min	Tritiated isopropyl groups per erythrocyte
"0"	1.5×10^7
60	5.0×10^7

Table 2. Survival of lymphocytes treated with *i*-PMS at room temperature expressed in per cent as shown by vital staining.

Treatment time min	Concn. of *i*-PMS %	0	0.02	0.1	0.4	1.0
1 + centrifugation		97	86	86	77	77
10 + ,,		97	86	82	76	70

investigate the sensitivity of these cells to treatment with *i*-PMS. The results are summarized in Table 2. In this experiment, pH was kept within the limits of 6.5—7.5 by the addition of tris buffer.

In accordance with the observations mentioned here, we propose the following procedure as a convenient method for tritium labelling of blood cells:

Tritium labelled isopropyl methanesulfonate (synthesis according to Wachtmeister *et al.*[5] from isopropanol-1,3-T from the Radiochemical Centre, Amersham) is dissolved while stirring in ice-cooled water and this solution is immediately mixed with a cell suspension in an isotonic buffer solution at pH 7—8. As soon as the mixing is complete the sample is centrifuged, and after discarding the supernatant the cells are washed 3—4 times with the isotonic buffer solution. All operations must be performed in a well-ventilated hood (or preferably a glove-box) as *i*-PMS is in itself highly toxic.

Measurement of the β-activity of individual cells is conveniently performed by microautoradiography. In bulk the activity is measured either in a liquid scintillation counter after hydrolysis of the propyl groups in alcoholic KOH and distillation of the released isopropanol together with the inactive ethanol, or in a window-less proportional counter after evaporation of the suspension on metal dishes. The hydrolysis—scintillation method yields the most reproducible values and although it may seem to be rather laborious, it is of great use if several samples are processed simultaneously. As mentioned above *i*-PMS is toxic, but no additional harmful effect on cells has been observed when the labelling is performed as described here. For example, labelled erythrocytes show no hemolysis and no change in the agglutinability with antiserum.

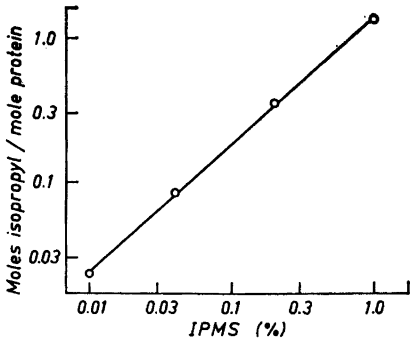

Fig. 1. Labelling of serum albumin by complete reaction with *i*-PMS of different concentrations.

i-PMS may be utilized for the labelling of proteins in solution as well as of cells. Fig. 1 shows the different degrees of labelling of bovine serum albumin corresponding to various concentrations of *i*-PMS. The stability of the label was tested and found to be equal to the stability of the ethyl label described earlier,[1] *i.e.* for pH values below about 8.5 at least 90 % of the isopropyl groups remain attached to the protein after incubating for 72 h at 37°C.

The advantage of using the rapidly reacting *i*-PMS instead of EMS in the labelling of living cells is obvious. For the labelling of antibodies and other proteins, however, it might in some cases be more convenient to use EMS. Due to its greater stability in water solution this substance is easier to handle and yields more reproducible degrees of labelling.

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