3. RESULTS AND DISCUSSION

a. Identification of adducts with DNA

As mentioned above, the chain of events leading to a monoclonal antibody has to be initiated by identification of the adduct against which to generate an antibody. Work was started on sulfur mustard adducts to DNA, because more experience was available with DNA adducts than with protein adducts. Part of the procedure for identification of DNA adducts is shown in Scheme 2. After introductory experiments with purified double-stranded DNA from calf thymus in aqueous phosphate buffer, fresh human blood from volunteers was used.

HUMAN BLOOD TREATED WITH 0.05 - 1mM [35 S] - MUSTARD

DNA

Nuclease P1 Deoxyribonuclease

NUCLEOTIDES

Alkaline phosphatase, type III

NUCLEOSIDES

Depurinate at 100°C

HPLC ANALYSIS

Scheme 2 Work-up and analysis of sulfur mustard adducts with DNA in white blood cells of human blood.

It was incubated with ≤ 1 mM radioactive sulfur mustard. DNA was isolated from the blood cells and broken down enzymatically to nucleotides and nucleosides. The alkylated purine bases were released from the latter and analyzed with