Frequencies of affected people and carriers among various ethnic groups in Israel

Ethnic groups	No. of randomly investigated individuals	No. of affected affected individuals	Frequency of affected individuals (%)	Frequency of carriers (2 pq) (%)	Frequency of genes q	±SE pq/N
Jewish						
Yemenites	11,212	44	0.39	11.7	0.0626	0.0016
Iraqi, Iranian	7,746	13	0.17	7.9	0.0410	0.0016
North Africans	10,532	26	0.25	9.5	0.0500	0.0015
Ashkenazi	33,975	3	0.008	0.02	0.009	0.003
Arabs	1,182	2	0.17	7.9	0.0411	0.0041

parents were unaffected, as were those of the individuals with complete absence of eosinophilic peroxidase. In no instance were the 2 types of deficiencies observed in the same families.

It is not certain whether a mutation of a regulatory gene is responsible for the intermediate activities of peroxidase[3]. Additional possibilities related to structural aspects of the enzyme need further investigation. Using chemical methods for more accurate characterization of enzyme properties and activities, one can expect to identify additional mutant alleles and eventually to detect sporadic cases of double heterozygous for different mutant alleles.

So far, no deleterious effects attributable to the lack of eosinophilic peroxidase could be observed in affected individuals and it seems that they are able to react with increased eosinophilic counts, as seen by us in several patients.

Recently Presentey[10] showed that the eosinophilic anomaly was expressed also at the ultrastructural level, by an enlargement of the core of the granules, and by an extreme thinning of the cortex, which is known to be the site of peroxidase activity in normal eosinophils.

The importance of the eosinophilic anomaly described[4,5] resides in its usefulness as an additional genetic marker for the study of population genetics. It parallels to some extent the distribution of other mutant genes, like G-6PD deficiency and Thalassemia, common to populations of the Mediterranean area. At the historical-geographical level, the continuation of these studies, especially among non-Jewish populations of this area, may help to trace affinities between different ethnic groups as well as to explain variety within populations. It should be mentioned in this context that the staining method of Undritz used by us is technically very simple and can be substituted for the standard methods used for staining of peripheral blood smears, thus allowing the detection of the anomaly while performing routine differential counts.

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Liposomes as immunological carriers for the preparation of antimannosyl antibodies

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Summary. Antiserum was raised against an aminophenyl derivative of D-mannose grafted on to a liposomal surface. As characterized by immunodiffucion, quantitative precipitation and hapten inhibition, the antiserum was found to contain mannose specific antibodies in addition to antibodies against the aromatic phenyl group.

The production of antibodies towards saccharide haptens generally requires the conjugation of saccharides with carrier proteins like bovine serum albumin or keyhole limpet hemocyanin[2,3]. In this communication we describe a new method for eliciting anticarbohydrate antibodies using liposomes as carriers for the monosaccharide, D-mannose, thereby eliminating the need for removing carrier-specific antibodies since liposomes themselves are known to be poorly immunogenic[4].

Methods. D-Mannose was covalently coupled to multilamellar phosphatidylethanolamine liposomes (egg lecithin:cholesterol:dicetyl phosphate:phosphatidylethanolamine = 7:2:1:2, molar ratio) as p-aminophenyl-α-glycoside using glutaraldehyde as the coupling reagent as described earlier[5]. Mannosylated liposomal suspensions (30 mg lipid/ml and 6 mg sugar/ml) were emulsified with an equal volume of complete Freund's adjuvant and 2 ml of the emulsion was injected into the hind food pads of a single rabbit. Three injections were given at every 10 days of which the last 2 were given in incomplete Freund's adjuvant. 7 days after the last injection, antiserum was collected by cardiac puncture. For immunological characterization of the antiserum, synthetic conjugates were prepared by coupling p-aminophenyl-α-D-mannopyranoside and p-aminophenyl-β-D-galactopyranoside with bovine serum albumin (BSA) through water soluble 1-ethyl-3-(dimethylaminopro-

pyl)-carbodiimide hydrochloride[6]. The mannoside and galactoside-BSA conjugates were found to contain 65 and 50 moles of sugar residues respectively (determined by the phenol-sulfuric acid method[7]) per mole of protein (Folin assay[8]) assuming a mol.wt of 67,000 for BSA. Quantitative precipitin studies were carried out by taking increasing amounts of these synthetic conjugates in duplicate tubes containing 10 µl antiserum and PBS (10 mM phosphate buffer, pH 7.4, 150 mM NaCl) in a final volume of 250 µl. After preliminary incubation at 37 °C for 1 h the tubes were incubated at 4 °C for 4 days with gentle agitation daily. The precipitates were then washed with 200 µl PBS (3 times) and the amount of protein in the precipitates was then estimated by Folin assay[8]. For hapten inhibition studies various ligands (50 mM) were incubated with 10 µl antiserum at room temperature for 1 h and then 100 µg of mannoside-BSA conjugate was added and incubated further at room temperature for 1 h followed by incubation at 4 °C for 4 days. Precipitates were washed as above and the amount of protein in the precipitates was estimated.

Results and discussion. The antiserum raised against α-mannosideliposomes was found to give an intense band of precipitation in Ouchterlony immunodiffusion with p-aminophenyl α-mannoside-BSA conjugate (fig. 1). No precipitation was observed with BSA as such. When the antiserum was characterized by quantitative precipitin studies with synthetic conjugates it was found that 124 µg of protein were precipitated by 100 µg of homologous conjugate (mannoside-BSA) with 10 µl antiserum whereas the same amount of heterologous conjugate (galactoside-BSA) could precipitate only 76 µg of protein (fig. 2). These suggest that apart from antibodies against the aromatic aglycone portion of the introduced hapten, the antiserum contains antibodies specific for mannose residues. The sugar specificity of the antiserum was further examined by the inhibitory capacity of various saccharide ligands in quantitative hapten inhibition studies (table). p-Aminophenyl-α-D-mannopyranoside was found to be the most potent inhibitor of the antiserum interaction with mannoside-BSA conjugate followed by p-aminophenyl-β-D-galactopyranoside, methyl-α-D-mannoside and D-mannose. The seemingly lower inhibitory potency (33%) of the parent ligand, p-aminophenyl-α-D-mannoside is not surprising considering the fact that a large number of haptenic groups are present per molecule of protein in synthetic conjugate (65 moles of sugar residues per mole of BSA in mannoside-BSA conjugate). The observed inhibition of the antiserum binding with mannoside-BSA by p-aminophenyl-β-D-galactose but not by galactose or lactose suggests that the antiserum recognizes only the aromatic phenyl moiety of the aminophenyl-galactose ligand but not the carbohydrate determinant. The contribution of the aromatic aglycone moiety i.e. the phenyl part of the introduced hapten to the specificity of antibody is also indicated by the ability of the antiserum to react with heterologous BSA-conjugate (galactoside-BSA) and by the higher inhibitory activity of p-aminophenyl-mannoside in comparison to D-mannose or methyl-α-D-mannoside. Finally, the ability of methyl-α-D-mannoside and D-mannose to inhibit the interaction of the antiserum with homologous-BSA conjugate clearly demonstrates the presence of antibodies directed against the sugar portion of the introduced hapten in liposomes. In addition to their already reported adjuvant effect[9,10], liposomes, therefore, may also be used as effective carriers for raising antibodies against saccharide determinants.

Percent inhibition of antimannosyl antiserum precipitation with mannoside-BSA conjugate by various ligands

Ligands	Inhibition (%)
p-Aminophenyl-α-D-mannopyranoside	33
p-Aminophenyl-β-D-galactopyranoside	22
Methyl-α-D-mannopyranoside	20
D-Mannose	15
D-Galactose	0
Lactose	0
D-Glucose	0
N-Acetyl-D-glucosamine	0
Melibiose	0

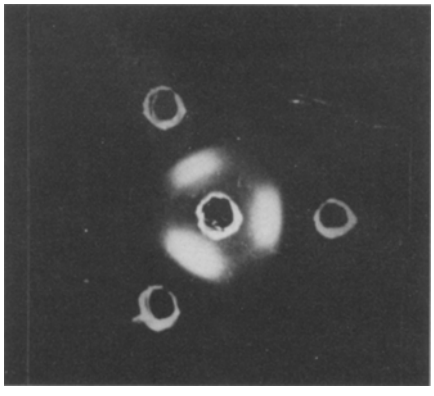

Figure 1. Immunodiffusion of antimannosyl antiserum against mannoside-BSA. Antiserum was added in the central well and the peripheral wells contain the synthetic conjugate.

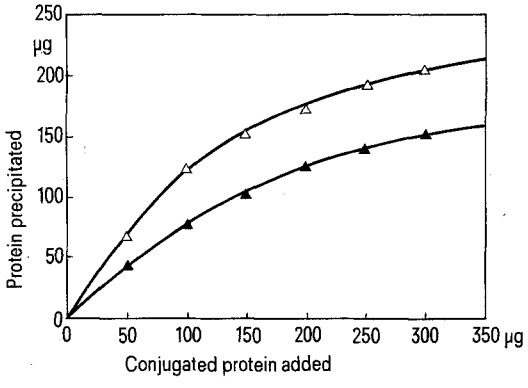

Figure 2. Quantitative precipitation curves of antiserum with carbohydrate-BSA conjugates. Antimannosyl antiserum precipitation with mannoside-BSA (△——△) and galactoside-BSA (▲——▲).

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