Enhanced stability of meningococcal polysaccharide vaccines by using lactose as a menstruum for lyophilization *

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A comparison is made between lactose and mannitol as additives for the lyophilization of meningococcal polysaccharide vaccines. From the stability data obtained on storage at high temperatures, it is concluded that vaccines containing lactose as a menstruum for lyophilization are much more stable than vaccines prepared with the currently used additive, mannitol. The enhanced stability of these vaccines makes it possible to store also group A vaccines at 5°C instead of at −20°C, and to use them in places without freezing facilities.

During the last few years it has been well established that group A and group C meningococcal polysaccharides can serve as protective antigens in man (see *9* for a survey). Group A vaccine has been successfully used for controlling epidemics in Brazil and Finland, and both group A and group C vaccines are now coming into general use.

One of the problems met in the production of these polysaccharide vaccines is the apparent instability of group A polysaccharide—as exemplified by a group A vaccine that afforded no protection at all in a field trial in Nigeria in 1969 (*6*, *10*): vaccine samples returned from the field showed a considerable decrease in the molecular weight of the polysaccharide (*10*), probably owing to unfavourable local temperatures. Since that time, group A polysaccharide has been thought to be intrinsically unstable; hence in accordance with the WHO Requirements for Meningococcal Polysaccharide Vaccine this group should be stored at −20°C or below (*9*). Group C polysaccharide, for which such a deterioration in the field has not been observed, is thought to be more stable, and can be stored, according to WHO Requirements, at 5°C or below. The storage of group A vaccine, and of combined group A plus group C vaccine, at −20°C raises considerable problems for producers, during transport of the vaccines, and most of all for consumers of the vaccines at places without adequate cold-storage facilities.

Some of the vaccines produced at the Rijks Instituut voor de Volksgezondheid, Bilthoven, with lactose as an additive for lyophilization seemed to be more stable than those prepared with the commonly used additive, mannitol. Therefore, the present study was undertaken to compare the effect of these additives on the stability of the vaccines at high temperatures.

MATERIALS AND METHODS

Polysaccharides

Group A, B, C, and W135 polysaccharides were isolated from the centrifuged culture fluid of 40–140-litre cultures of the appropriate strains of *Neisseria meningitidis*, grown for 10–20 h in Frantz's medium (*2*) supplemented with 2 g/litre of yeast extract dialysate. After the isolation procedure, including cold-phenol extraction, described by Gotschlich et al. (*3*, *4*, see also *9*), the purified polysaccharides were dried at 20°C *in vacuo* over P_2O_5. The dried polysaccharides were stored at −20°C or below.

Vaccines

Vaccine lots No. 1–6 were produced by dissolving the polysaccharides at a concentration of 250 mg/litre each in solutions consisting of 50 g of α-lactose (Sigma, lot No. 14C-1650) or of D(−)mannitol (Merck, lot No. 493282) per litre. The vaccines were dispensed in 2.2-ml quantities into 12-ml vials,

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which were closed *in vacuo* with rubber caps after lyophilization. Lyophilization conditions in the Leybold-Heraeus GT-25 apparatus were: primary drying for 7 h at a constant pressure of approximately 5.3 Pa, condenser temperature −65°C, immediately followed by secondary drying over P_2O_5 for 10 h, during which period the pressure was reduced to about 1.33 mPa by means of an oil-diffusion pump. At no stage during the lyophilization process did the temperature of the product exceed 20°C. Lots No. 1, 2, and 3 contained thiomersal to give a final concentration of 0.1 g/litre after reconstitution with 2.2 ml (lot No. 1) or 5.5 ml (lots No. 2 and 3) of saline; lots No. 4, 5, and 6 did not contain thiomersal.

Vaccines AM, AL, CM, and CL were produced by dissolving the purified group A (lot No. MU 59) or group C (lot No. MU 77) polysaccharides at a concentration of 1.25 g/litre in 5% lactose (AL, CL) or mannitol (AM, CM) solution; 2-ml quantities were filled into 12-ml vials and lyophilized.

Residual moisture in the lyophilized vaccines, determined as weight loss at 56°C *in vacuo* over P_2O_5, was 0.6–1.1%. The vaccines were kept in stock at −20°C.

Gel filtration

To detect changes in molecular weight of the polysaccharides, gel filtration was carried out on Sepharose 4B (Pharmacia lot No. 7137 or 3325) at 5°C in 0.2 mol/litre NaCl, 0.01 mol/litre Tris/HCl, pH 7.4, flow rate 20–25 ml/h, with monitoring of the column eluates with a Uvicord III[a] at 206 nm. The column (50 cm long and 2.5 cm in diameter) was loaded with the contents of one vial of vaccine dissolved in 1 ml of elution buffer. Void volume (V_o) and total volume (V_t) were determined with blue dextran and sodium azide, respectively. K_D volumes were calculated from the elution volume V_e according to $K_D=(V_e-V_o)/(V_t-V_o)$. The amounts of polysaccharide eluted at the void volume, or before $K_D=0.50$, were estimated from the recorder tracings of the column eluates.

Enzyme-linked immunosorbent assay

The antigenicity of the polysaccharides was tested in the enzyme-linked immunosorbent assay (ELISA), using the following sequence of steps, separated by washings with phosphate-buffered saline (PBS) +0.01% Tween-80:

[a] LKB-Produktar, Bromma, Sweden.

(*a*) Coating of polystyrene tubes (LKB) with the Ig fraction of sheep anti-A or anti-C serum (32 mg/litre in PBS, 16 h at room temperature);

(*b*) Incubation with antigen (25 μg/litre in PBS+0.05% Tween 80, 2 h at 37°C);

(*c*) Reaction with the Ig fraction of sheep anti-A or anti-C serum, conjugated (*5*) with horseradish peroxidase (Sigma, type VI) (diluted in PBS+0.01% Tween 80+0.5% bovine serum albumin, 2 h at 37°C);

(*d*) Incubation with 2-hydroxy-5-aminobenzoic acid and H_2O_2 (*8*) for 10 min at room temperature, and measurement of the increase in optical density at 449 nm.

The antisera used in this test were prepared by hyperimmunization of sheep with whole bacterial cells (*1*); they contained mainly antibodies to the polysaccharide antigens.

RESULTS

Table 1 gives the results of gel filtration after storage at 35°C of dissolved group A and group C vaccines with lactose. These data show that group A polysaccharide in solution is not necessarily unstable. After storage for 48 h, both group A and group C vaccines still complied with the WHO Requirements as regards K_D value and the amount of polysaccharide eluted with a K_D value less than or equal to 0.50.

Table 2 gives the main results of gel filtration of vaccines, stored in lyophilized form for one month at 35°C or 50°C, in comparison with material stored

Table 1. Gel filtration on Sepharose 4B of vaccines stored at 35°C after solution in 1 ml of elution buffer

Vaccine (lot No. and group)	No. of hours at 35° C	Material eluted at void volume (%)	Material eluted before K_D = 0.50 (%)	Peak K_D material not excluded
1 A	0	85	90	0.23
1 A	6	90	95	0.23
1 A	24	30	90	0.29
1 A	48	35	85	0.32
1 C	0	40	90	0.30
1 C	24	20	75	0.35
1 C	48	25	75	0.32
WHO Requirements		—	⩾ 65	⩽ 0.40

Table 2. Gel filtration on Sepharose 4B of vaccines stored in the lyophilized state for one month at 35°C or 50°C, compared with vaccines kept at —20°C

Vaccine (lot No. and group)	Menstruum for lyophilization	Material eluted at void volume (%)			Material eluted before K_D = 0.50 (%)			Peak K_D material not excluded		
		−20°	35°	50°	−20°	35°	50°	−20°	35°	50°
1 (A + C)	lactose	35	45	30	85	90	85	.26	.21	.31
2 (A + C)	lactose	20	30	25	85	85	80	.28	.33	.35
3 (A + C)	mannitol	50	40	5	85	60	15	.29	.31	.63
4 C	mannitol	55	25	15	85	85	80	.33	.33	.33
5 B	mannitol	70	25	20	90	75	55	.42	.45	.51
6 W135	mannitol	100	65	20	100	95	80	(absent)	.23	.27
WHO Requirements			—			⩾ 65			⩽ 0.40	

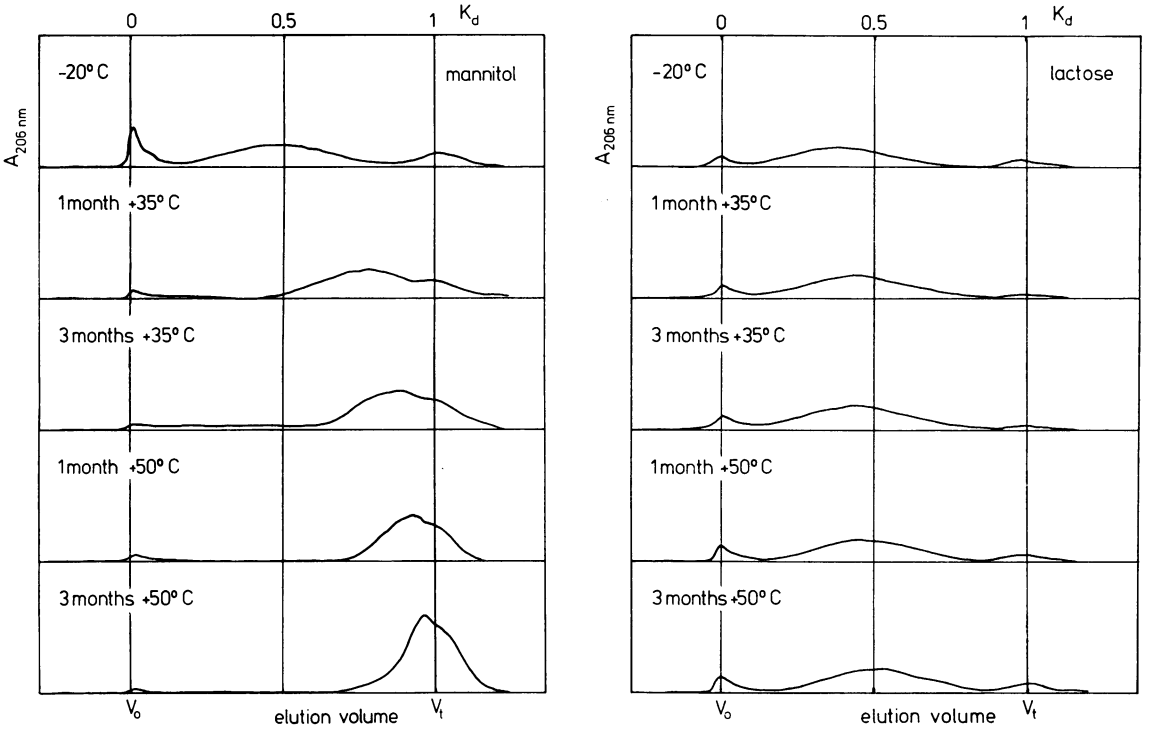

Fig. 1. Elution profiles on Sepharose 4B gel filtration of group A vaccine after storage for 1 or 3 months at a high temperature. Left-hand part, vaccine AM (mannitol); right-hand part, vaccine AL (lactose).

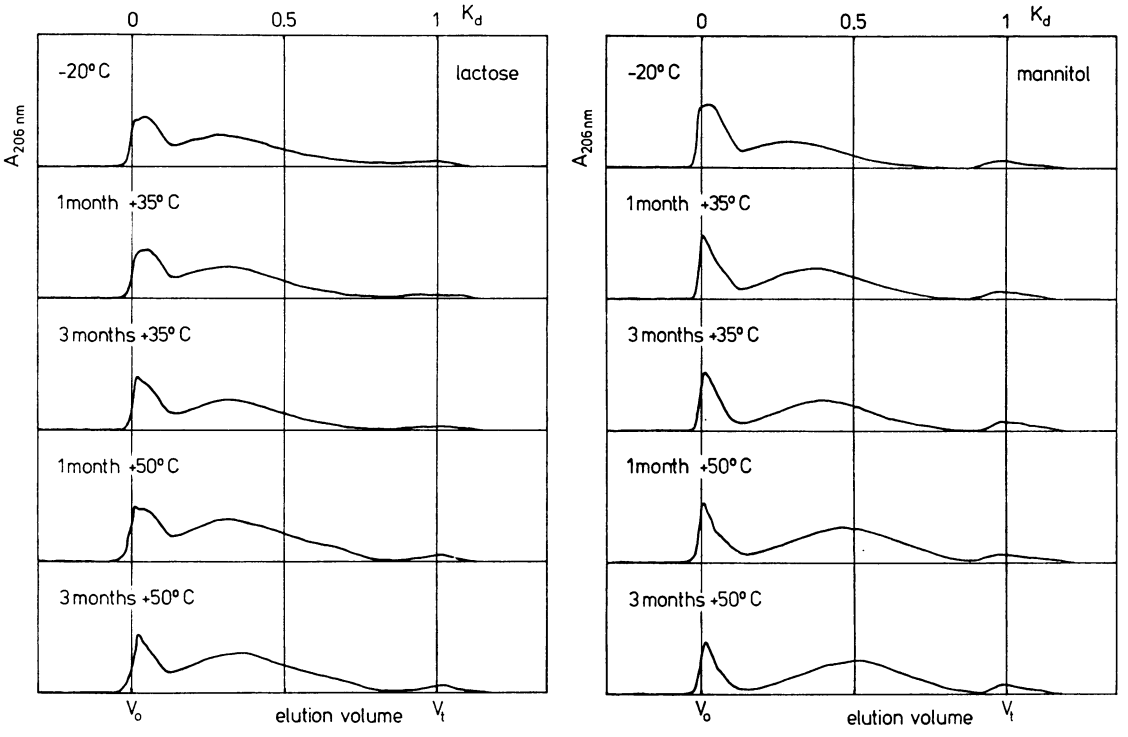

Fig. 2. Elution profiles on Sepharose 4B gel filtration of group C vaccine after storage for 1 or 3 months at a high temperature. Left-hand part, vaccine CM (mannitol); right-part hand, vaccine CL (lactose).

at −20°C. For vaccines lyophilized with lactose (lots No. 1 and 2), neither the amount of high molecular weight polysaccharide excluded from the gel matrix nor the amount of material eluted with a K_D value less than or equal to 0.50 is significantly affected by storage at elevated temperatures. For vaccines lyophilized with mannitol, however, the amount of excluded material decreases appreciably, with a concomitant increase in the amount of material of lower molecular weight. At the same time, there was a decrease in the amount of polysaccharide eluted with a K_D value less than or equal to 0.50.

From the recorder tracings of the elution profiles of the combined vaccines (group A+C) no discrimination can be made between the contributions of the separate polysaccharides. Therefore, in another experiment with these polysaccharides, the stabilizing effects of the additives mannitol and lactose were compared. The resulting chromatograms, obtained with vaccines AM, AL, CM, and CL, stored at −20, +35, and +50°C, are shown in Fig. 1 and 2. From Fig. 1 it can be seen that group A vaccine lyophilized with lactose is much more stable than the corresponding vaccine with mannitol. Fig. 2 shows that group C vaccine, too, has higher stability when

Table 3. Antigenicity of group A and group C meningococcal polysaccharide vaccines after storage at high temperatures, as determined with the ELISA technique. Values are expressed as percentages of the optical density changes found with vaccines stored at —20°C

Storage temperature (° C)	Storage time (months)	Vaccine			
		AM	AL	CM	CL
−20	–	100	100	100	100
35	1	67	93	91	97
35	3	64	97	90	95
50	1	67	92	90	97
50	3	45	92	83	98
Control (PBS)		6	6	5	5

lyophilized in the presence of lactose instead of mannitol.

The decrease of antigenicity with storage of the group A–mannitol vaccine at elevated temperature, and to a lesser extent of the group C–mannitol vaccine, is shown in Table 3, which contains the results of an ELISA determination on material stored at a high temperature. This phenomenon was not observed when lactose was used as an additive. These results agree well with the elution profiles shown in Fig. 1 and 2. Moreover, in an Ouchterlony double-diffusion experiment (not shown), the group A–lactose vaccine, after 3 months' storage at 50°C, was still identical with the vaccine stored at −20°C, without changes in the position of the precipitation lines, whereas the group A–mannitol vaccine after only one month at 35°C no longer showed any precipitation.

DISCUSSION

Lactose is a major constituent of skimmed milk, which has been in use for many years with good results in our institute and many others for the lyophilization of bacterial strains. It is obvious from the stability data presented in this paper that, at least in the case of group A and group C polysaccharide vaccines, lactose is preferable to mannitol as the menstruum for lyophilization. However, we can offer no explanation why lactose gives better results than mannitol. Moisture determinations have yielded values of about 1% with either additive, indicating that differences in residual moisture probably do not account for the differences in stability.

Storage of vaccine samples for one month at 50°C may give a good indication how these vaccines will behave at unfavourable temperatures, such as may be encountered in tropical areas; in this way, the failure of a vaccination programme may be prevented.

The present data make it highly probable that group A vaccine, like group C vaccine, may be stored at 5°C without any change in molecular weight if lactose is used as a stabilizer, thus facilitating storage and transport of the vaccines.

For group C vaccines, lactose has one minor disadvantage: it interferes with the determination of the sialic acid content of the vaccine, according to Svennerholm (*7*). Such interference, however, can easily be overcome by dialysis of the vaccine prior to the sialic acid determination.

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RÉSUMÉ

ACCROISSEMENT DE LA STABILITÉ DES VACCINS ANTIMÉNINGOCOCCIQUES POLYOSIDIQUES GRÂCE À L'UTILISATION DU LACTOSE COMME MILIEU DE LYOPHILISATION

Le vaccin antiméningococcique polyosidique de groupe A, préparé actuellement avec du mannitol comme milieu de lyophilisation, a l'inconvénient d'être instable aux températures ambiantes; c'est pourquoi, selon les normes OMS relatives au vaccin antiméningococcique polyosidique, ce dernier doit être conservé à une température égale ou inférieure à −20°C, ce qui constitue une sérieuse complication pour les producteurs de vaccin, au cours du transport, ainsi que pour les utilisateurs là où les moyens de congélation font défaut.

La présente communication fournit des données selon lesquelles le vaccin du groupe C, lyophilisé avec du mannitol, subit également une certaine détérioration au cours du stockage à des températures élevées. Or, le remplacement du mannitol par un autre corps (lactose) permet d'obtenir des vaccins de groupe A et de groupe C qui restent bien plus stables lors de la conservation à ces températures. Cette stabilité accrue offre la possibilité de conserver aussi les stocks de vaccin de groupe A à 5°C au lieu de −20°C. En outre, elle diminue le risque de voir des vaccins, conformes aux normes de l'OMS au moment de leur mise en circulation par le fabricant, perdre leur immunogénicité du fait de hautes températures telles qu'il s'en rencontre par exemple dans les régions tropicales.

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