further studies of serum proteins in pregnancy utilizing more refined zone electrophoretic methods would produce useful information. The use of immunoelectrophoretic techniques is particularly indicated.

Summary. During pregnancy, total serum protein and serum albumin concentration decreased, while the α- and β-globulin protein fractions increased and the γ-globulin protein fraction remained at a rather constant level throughout pregnancy. Total serum glycoprotein, expressed as protein bound hexose, increased throughout the gestational period. This was accompanied by a rise in the levels of all glycoprotein fractions expressed as mg per 100 ml. Relative to the protein content of each fraction, the glycoprotein content of the albumin and γ-globulin fractions increased during pregnancy, while the α_1-, α_2- and β-fractions decreased in relative percentage of bound carbohydrate. During the early post partum period, albumin and γ-globulin glycoprotein decreased, while α_1-, α_2- and β-globulin glycoprotein increased. These results indicate that striking alterations of plasma protein metabolism occur in pregnancy.

1. Lustig, B., Novak, J., *Exp. Med. Surg.*, 1946, v4, 252.

2. Shetlar, M. R., Kelly, K. H., Foster, J. V., Shetlar, C. L., Everett, M. R., *Am. J. Obst. and Gynec.*, 1950, v59, 1140.

3. Stary, Z., Bursa, F., Teezok, O., Cindi, R., *Hoppe-Seylers Z. Physiol. Chem.*, 1955, v288, 55.

4. Mack, H. C., *The Plasma Proteins in Pregnancy*, Charles C Thomas, Publisher, Springfield, Ill., 1955.

5. Shetlar, M. R., Cahill, C., Stidworthy, G., Shetlar, C. L., PROC. SOC. EXP. BIOL. AND MED., 1956, v93, 44.

6. Shetlar, M. R., Foster, J. V., Everett, M. R., *ibid.*, 1948, v67, 125.

7. Stidworthy, G., Payne, R. W., Shetlar, C. L., Shetlar, M. R., *J. Clin. Invest.*, 1957, v36, 309.

Received January 23, 1963. P.S.E.B.M., 1963, v112.

Effect of pH on Thermal Stabilization of Oral Poliovirus Vaccine by Magnesium Chloride. (28201)

JOSEPH L. MELNICK AND CRAIG WALLIS

Department of Virology and Epidemiology, Baylor University College of Medicine, Houston, Texas

Polioviruses(1) and other enteroviruses(2) are stabilized at elevated temperatures in high ionic concentrations of $MgCl_2$. In molar $MgCl_2$ at 50°C, poliovirus is stabilized for at least 2 hours; at 37°C for 3 days; and at room temperature (25°-28°C) for 21 days. This report is concerned with the role of pH in the stabilization of live poliovirus vaccines with $MgCl_2$, and the effect of long term (18 months) storage at 4°C.

Materials and methods. Oral poliovirus vaccines (type 1, LSc; type 2, P712; and type 3, Leon) were kindly provided by Wyeth Laboratories.

For virus assay, kidneys from immature rhesus monkeys were trypsinized and grown in lactalbumin hydrolysate medium containing 0.2 mM $AlCl_3$ to suppress adventitious agents(3). Cultures were maintained in the same M-E medium but free of Al ions. Titrations were carried out by determination of plaque-forming units (PFU), using bottle cultures, with 25 mM $MgCl_2$ in the agar-nutrient medium to expedite plaque counting(4).

Results. Effect of pH on stabilization by $MgCl_2$. On occasion, samples of poliovirus in molar $MgCl_2$ have failed to stabilize the virus at 28°C to the extent expected. In these instances the caps on vaccine vials were found to be loose, and an increase in pH was evident in the samples in question. To determine the effects of long term exposure to the atmosphere, types 1, 2, and 3 poliovirus vaccines were diluted to contain 10^7 PFU/ml in M $MgCl_2$ and in M-E medium, respectively. The pH of the vaccines in M $MgCl_2$ was 6.4 at time of mixing, but after 24 hours the pH equilibrated to 6.8-7.0 and remained constant for 30 days at 28°C

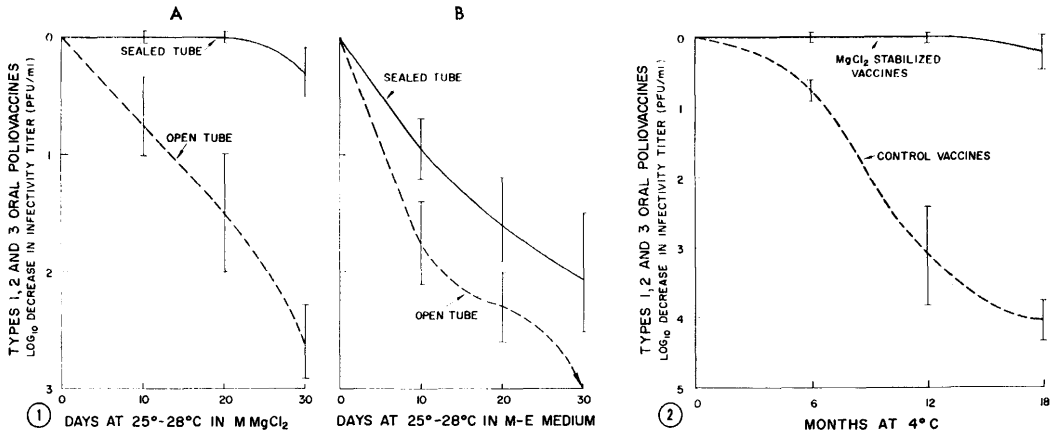

FIG. 1. Thermal resistance of $MgCl_2$-stabilized poliovirus vaccine in tightly stoppered vials. Type 1, 2, and 3 vaccines were diluted to contain 10^7 PFU/ml in (A) M $MgCl_2$ and (B) M-E medium. Vaccines in tightly stoppered and loosely capped vials were stored at room temperature (25°-28°C) for 30 days.

FIG. 2. Effect of prolonged storage of poliovirus vaccines at 4°C. Type 1, 2, and 3 oral poliovirus vaccines were diluted to contain 10^7 PFU/ml in M $MgCl_2$ ($MgCl_2$-stabilized vaccines) and in M-E medium (control vaccines). All samples were stored in tightly stoppered vials.

in tightly stoppered vials. In loosely capped vials the pH of the vaccine samples in $MgCl_2$ was elevated to 7.6-7.8 after 24 hours, and varied between 7.8 and 8.1 over a period of 30 days at 28°C. The vaccines mixed with M-E medium were at pH 7.4 at time of mixing, and in tightly stoppered vials attained a range of pH 7.6-7.8 during the 30 days at 28°C. When loosely capped vials were used, these samples showed pH increases at 24 hours to 8.5-8.8 and remained within this range throughout the 30 day period.

Fig. 1 shows the results of a typical experiment with these vaccines stored at room temperature (25-28°C) for 30 days in tightly stoppered and in loosely capped vials. The $MgCl_2$-stabilized vaccines in tightly stoppered vials manifested no loss of infectivity for 20 days at this temperature. At 30 days the infectivity titer dropped only 0.1-0.5 $\log_{10}$. The same samples in loosely capped vials lost infectivity rapidly. The unstabilized samples in tightly stoppered vials, in contrast to the $MgCl_2$-stabilized vaccines, lost significant infectivity at 10 days at 25-28°C, 0.7-1.2 $\log_{10}$; at 20 days, 1.2-1.9 $\log_{10}$; and at 30 days, 2.3-2.8 $\log_{10}$. Similarly, the corresponding samples in M-E medium in loosely capped vials lost infectivity even more rapidly than those in the sealed vials. These results indicate that vaccines should be stored in tightly stoppered vials.

To determine the optimum pH at which $MgCl_2$-stabilized vaccines should be maintained to achieve minimal loss of infectivity, the following experiment was performed. Types 1, 2, and 3 oral vaccines were mixed with equal volumes of 2M $MgCl_2$ to contain 10^7 PFU/ml. The pH of this mixture was 6.4. Aliquots were then adjusted with HCl to pH 5.0 and 6.0 and with NaOH to pH 7.0. Vials were tightly stoppered and stored at room temperature. Similarly, control samples at 10^7 PFU/ml in M-E medium (pH 7.4) without added $MgCl_2$ were adjusted with HCl to pH 5.0, 6.0, and 7.0, and also stored in tightly stoppered tubes. The pH changes of these samples on storage were followed for 20 days, as shown in Table I, together with the pH changes in loosely capped vials containing test and control samples whose initial pH had not been adjusted before storage. All vials showed increases in pH, but again these increases were much more marked in the loosely capped vials than in those which were tightly stoppered. Only the results with type 1 are shown; the findings with types 2 and 3 were similar.

The losses of infectivity titers of stabilized and unstabilized vaccines stored in tightly

TABLE I. pH Levels of Virus Samples After Storage.*

Test mixture	Vials	Initial pH	pH after storage at 25-30°C: 1 day	10 days	20 days
Vaccine + 2M $MgCl_2$	Tightly stoppered	5.0†	5.8	5.8	5.8
		6.0†	6.5	6.7	6.5
		6.4§	6.8	6.8	6.9
		7.0‡	7.4	7.5	7.5
	Loosely capped	6.4§	7.8	7.9	7.8
Vaccine + M-E medium	Tightly stoppered	5.0†	5.6	5.8	5.8
		6.0†	6.8	6.8	6.9
		7.0†	7.4	7.4	7.4
		7.4§	7.6	7.7	7.7
	Loosely capped	7.4§	8.8	8.8	8.7

* Type 1 poliovirus (oral) vaccine was mixed with an equal volume of 2M $MgCl_2$ and pH levels adjusted as shown above. After 1, 10 and 20 days pH levels were again tested. Control samples consisted of equal volume of vaccine and M-E medium.

† Adjusted with HCl.

‡ Adjusted with NaOH.

§ Not adjusted—represents pH after mixing equal volume of vaccine and 2M Mg^{++} or M-E medium as indicated.

stoppered vials under these various pH conditions, over a period of 30 days, are shown in Table II. It is evident that with $MgCl_2$-stabilized vaccine, storage in tightly stoppered vials at an initial pH of 6.4 is optimum at room temperature. To achieve this pH, the vaccine as manufactured in M-E medium is merely mixed with the $MgCl_2$ solution and then tightly stoppered. No adjustment of pH with HCl or NaOH is necessary.

Effect of prolonged storage of oral poliovaccines at 4°C. Types 1, 2 and 3 oral vaccines containing 10^7 PFU/ml were stored at 4°C in tightly stoppered vials in (a) lactalbumin hydrolysate M-E medium, and (b) in M $MgCl_2$, for a period of 18 months. Ali-

TABLE II. The Effect of pH on $MgCl_2$-Stabilized Vaccines at 25-28°C in Tightly Stoppered Vials.

Vaccine†	Initial pH*	Log_{10} decrease in infectivity titer (PFU/ml) due to inactivation: 10 days, Control vaccine	10 days, Stabilized vaccine	20 days, Control vaccine	20 days, Stabilized vaccine	30 days, Control vaccine	30 days, Stabilized vaccine
Type 1	5.0	2.0	.3	2.5	.8	2.9	1.0
	6.0	1.3	.0	2.0	.1	2.7	.8
	6.4	1.3	.0	1.5	.2	2.5	.5
	7.0	1.0	1.0	1.6	1.4	2.1	1.7
	7.4	1.2	—	1.9	—	2.5	—
Type 2	5.0	2.5	.2	2.8	.2	3.2	.9
	6.0	1.4	.0	1.8	.3	2.0	.7
	6.4	1.2	.0	1.7	.0	2.2	.5
	7.0	1.2	.7	1.5	.9	2.0	1.2
	7.4	1.0	—	1.4	—	1.8	—
Type 3	5.0	1.4	.0	1.8	.2	2.5	.5
	6.0	.8	.0	1.0	.0	1.3	.5
	6.4	1.2	.0	1.3	.0	1.5	.1
	7.0	1.0	.2	1.3	.4	1.4	.6
	7.4	.8	—	1.2	—	1.5	—

* pH represents adjusted levels of vaccines at time experiment was initiated, except for pH 6.4 in $MgCl_2$ and pH 7.4 in M-E medium, which were not adjusted. See Table I for pH drifts on storage.

† Vaccines diluted to contain $10^{7.3}$ PFU/ml in M-E medium and then mixed with an equal volume of 2M $MgCl_2$, or M-E (for "control vaccine"), respectively, to contain 10^7 PFU/ml as starting concentration of storage experiment.

quots were also stored frozen at −20°C. These were thawed and titrated on the same day that the experimental vials were titrated, as a control of the titration procedure. The deviations from the titers of the −20° samples are shown in Fig. 2. The titers of the −20° samples remained relatively constant during this period, varying within a few tenths of a $\log_{10}$ unit. Fig. 2 shows the stabilizing effects of $MgCl_2$ on the experimental samples stored at 4°C. After 6 months at 4°C, the ordinary vaccines had lost 75% infectivity, and at 12 months their infectivity had diminished 99 to 99.9%, whereas vaccines in $MgCl_2$ showed no detectable loss of infectivity after 12 months. After 18 months, the ordinary vaccines had lost 4.0 $\log_{10}$ infectivity (99.99%), while among the $MgCl_2$-stabilized samples type 2 virus had lost 0.4 $\log_{10}$ infectivity; type 3, 0.2 $\log_{10}$; and no detectable loss was shown in the type 1 samples.

Discussion. The practical application of $MgCl_2$ as a stabilizing vehicle for poliovirus vaccines is shown in the results of a large field trial of $MgCl_2$-stabilized vaccine in Israel, where 300,000 children were fed the $MgCl_2$-stabilized vaccine(5). The stabilized vaccine was shipped from the United States and taken to the field without refrigeration. The antibody response after feeding stabilized vaccine is similar to that obtained with ordinary vaccine kept frozen until immediately before use(6).

It is evident that $MgCl_2$-stabilized poliovirus vaccine can be used by the practicing physician or the health officer without the strict limitations placed on the ordinary vaccine. Present regulations in the United States require that the vaccine be kept frozen and that, once thawed, it be held at a temperature no higher than 10°C for no longer than 1 week, at which time it must be discarded.

From the results of the experiments described here, it is seen that $MgCl_2$-stabilized vaccines may be stored (in tightly stoppered vials) for over a year in the liquid state in the ordinary refrigerator without loss of infectivity. The advantages of $MgCl_2$-stabilized vaccines are manifold: a) Vaccines are stabilized against thermal inactivation. b) Because of the bacteriostatic and mycostatic effects of M $MgCl_2$, no other preservative is necessary. c) Refrigeration is unnecessary when vaccines are in transit in common carriers. d) Stabilized vaccines can be taken to the field (clinic, physician's office, etc.) and used without concern over refrigeration.

Summary. $MgCl_2$-stabilized oral poliovirus vaccines can be stored in the liquid state at 4°C for one year without detectable loss of infectivity, and for 18 months with only slight decreases in infectivity. Maximum stabilization is attained in M $MgCl_2$ at pH 6.4 in tightly stoppered vials. Under these conditions, vaccines of all 3 types are stable for at least 3 weeks at room temperature (25-28°C). The use of the stabilized vaccine eliminates the limitations imposed on the ordinary thermolabile vaccine.

1. Wallis, C., Melnick, J. L., *Texas Rep. Biol. and Med.*, 1961, v19, 409.

2. ———, *Virology*, 1962, v16, 504.

3. ———, *Texas Rep. Biol. and Med.*, 1962, v20, 465.

4. ———, *Virology*, 1962, v16, 122.

5. Yofe, J., Goldblum, N., Eylan, E., Melnick, J. L., *Am. J. Hyg.*, 1962, v76, 225.

6. Melnick, J. L., *Ann. N. Y. Acad. Sci.*, 1962, 101, 331.

Received January 24, 1963. P.S.E.B.M., 1963, v112.