support normal metabolic activity. In other words, the pituitary gland, particularly the anterior lobe, is profoundly influenced by ionizing rays. As a result of this radiosensitivity along with other disfunctions, a state of adrenal insufficiency is established. From this one can assume that the extent of pituitary damage determines the degree of adrenal insufficiency. The fact that both cortisone and DCA are able to increase the survival time in DBA mice suggests that in this strain a state of adrenal insufficiency is avoided either by rendering the pituitary less sensitive to ionizing radiation or by preventing an early decrease in adrenal-cortical activity after irradiation, which, if prolonged, may possibly result terminally in a relative adrenal insufficiency and finally death.

Summary. 1. Young male and female DBA mice were exposed to 500 r head radiation or whole-body radiation. A correlation is observed between head and whole-body radiation, as judged by the mortality rate. In both instances a 500 r dose gives a 100% mortality rate, but mice receiving whole-body radiation succumb sooner. 2. Mice receiving 500 r head radiation and primed before or after radiation with cortisone or desoxycorticosterone showed evidence that both of these compounds afford protection from the lethal effect of ionizing radiation. 3. Interpretations of the role of the pituitary-adrenal axis under the conditions of these experiments are discussed.

We wish to thank Dr. Kenneth Wade Thompson, Director of Medical Research, Organon Inc., for the generous donation of desoxycorticosterone acetate (Doca).

1. Perla, D., and Marmorston, J., *Natural Resistance and Clinical Medicine,* Little, Brown and Co., Boston, 1941.

2. Treadwell, A., Gardner, W. T., and Laurence, J. H., *Endocrinology,* 1943, v32, 161.

3. Patt, H. M., Straube, R. L., Tyree, E. B., Swift, M. N., and Smith, D. E., *Nuclear Science Abst.,* 1949, v3, 428.

4. Graham, J. B., and Graham, R. M., *Proc. Nat. Acad. Sc.,* 1949, v35, 102.

5. Selye, Hans, *Textbook of Endocrinology, Acta Endocrinologica,* Montreal, Canada, 1947.

6. ———, *Stress, Acta Endocrinologica,* Montreal, Canada, 1951.

7. Ellinger, F., PROC. SOC. EXP. BIOL. AND MED., 1947, v64, 31.

8. Straube, R. L., Patt, H. M., Tyree, E. B., and Smith, D. E., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 530.

9. Graham, J. B., Graham, R. M., and Graffeo, A. J., *Endocrinology,* 1950, v46, 434.

10. Smith, W. W., Smith, F., and Thompson, E. C., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 529.

11. Jacobson, Leon O., *Canc. Res.,* 1952, v12, 315.

12. Patt, H. M., Swift, M. N., Tyree, E. B., and John, E. S., *Am. J. Physiol.,* 1947, v150, 480.

Received September 30, 1952. P.S.E.B.M., 1952, v81.

Stability of Mumps Antibody and Antigen at Various Temperatures.* (19889)

A. G. BENNETT AND J. E. KEMPF.

From the Department of Bacteriology, University of Illinois College of Medicine, Chicago.

During the course of an investigation on the antibody response of man to vaccination with the mumps antigen it became necessary to obtain information as to the stability of the mumps antibody and antigen. The stability of the complement-fixing (CF) antibody of immune sera was tested in relation to temperature variations during inactivation prior to the CF test, and following storage. The stability of the CF antigen and the CF antibody-producing antigen of a commercially prepared vaccine were also tested.

Materials and methods. The complement-fixation test. The test employed in these studies was developed by Habel(1). Commercial hemolysin was used in the amount of 2 units in 0.2 ml. Lyophilized guinea pig comple-

* This study was supported in part by a grant from Eli Lilly and Co., Indianapolis, Ind.

ment† was diluted to contain 1⅓ units in 0.2 ml. Defibrinated sheep red blood cells (SRBC) were used as a 2% suspension in 0.85% NaCl solution; the salt solution also served as the diluent in the test. Depending upon which was being tested, serial 2-fold dilutions of the antigen or antibody were made, the lowest dilution being 1:2 in 0.2 ml unless otherwise mentioned. Four CF units of antigen or antiserum (depending on which was being titered) and 1⅓ units of complement, each in 0.2 ml, were then added to all tubes except the controls. The tubes were incubated for one hour in a waterbath at 37°C. After adding 0.2 ml of a 2% suspension of SRBCs plus 2 units of hemolysin in 0.2 ml, the tubes were again incubated at 37°C for one hour and placed in a refrigerator at 5°C; the results were read after 18 hours. The endpoint of the reaction was taken as the greatest initial dilution of test material causing 4+ fixation. All tests were run in duplicate. *Storage of stock antiserum.* The pooled mumps convalescent serum, which was obtained from the Michael Reese Serum Center, Chicago, was transferred aseptically to chemically clean glass ampules in approximately one ml amounts. The ampules were flame-sealed and stored at —15°C until tested. *Determination of the antigenicity of the vaccine.* In testing the antigenicity of the vaccine, 450 g male guinea pigs were employed. An adjuvant was used after the procedure of Freund (2). The proportions were: 2 parts of mumps vaccine, 1 part Falba, and 2 parts paraffin oil containing heat-killed *M. tuberculosis* mixed well immediately before injection by forcibly filling and emptying a syringe with the material. *Preparation of mumps vaccine.* The vaccine‡ was a suspension of an alcohol concentrated, ultra-violet light inactivated mumps virus (Enders and Habel strains) which had been grown in embryonated eggs. The antigen was suspended in 0.85% NaCl solution with "Merthiolate" (Thimerosal, Lilly) 1:10,000 and formalin 1:1,000 added as preservatives. Sterility and antigenicity tests were made according to specifications of the National Institutes of Health of the United States Public Health Service.

Results. Heat-stability of the mumps CF antibody. In performing the CF test the practice has been to inactivate the serum at 56°C to destroy the complement; however, it is now recognized that cross reactions with other antigens occur after inactivation at 56°C and that these can be eliminated by inactivation at 60°C. In an effort to determine the stability of mumps antisera at higher temperatures, samples were heated at different temperatures for varying periods of time. Five one ml samples of undiluted antisera were placed in waterbaths ranging from 48 ± 0.5°C to 64 ± 0.5°C in increments of 4°C for 30 minutes; one other sample remained at room temperature. In 4 experiments no decrease in CF titer was noted between 22° and 60°C. Heating the antisera for 30 minutes at 64°C coagulated them and rendered titration impossible.

The stability of the antibody on storage at —15°C. During the course of 2 months the sera were stored at —15°C in sealed ampules and the titer remained 1:80.

The effect of simulated shipping conditions on the CF titer of antisera. An effort was made to determine the effect of sending antisera from the point of collection to the laboratory upon the determination of antibody levels. One series of samples of antisera was refrigerated for 7 days in an insulated box containing dry ice, the temperature gradually rising from —35° to —27°C as the CO_2 sublimated. Three samples were stored unfrozen: one at 5°C to approximate cold weather conditions, one at room temperature (22° to 30°C) for moderate weather, and one series at 37°C to simulate warmer weather. All samples were titered each day during a period of 7 days. There was no loss in CF titer under these conditions. Those samples stored at room temperature and at 37°C became anticomplementary within one week, but this condition was corrected by inactivating the serum at 60°C for 25 minutes.

Having determined the stability of the antibody under the conditions tested, it seemed advisable to determine the effects of various

† Processed by Carworth Farms, New City, N. Y.

‡ Kindly supplied by Eli Lilly and Co., Indianapolis, Ind.

TABLE I.
Effect of Storage at Various Temperatures on Complement Fixation Titer of Mumps Vaccine.

	Reciprocal of mean titer				
	Temp., °C				
Time, wk	−70	−15	5	22 to 30	37
0	32	32	32	32	32
1	16	8	16	32	32
2	8	4	32	32	32
4	4	<2	32	16	32
5	2	2	32	16	16
6	2	<2	32	16	16
7	2	<2	32	16	16

periods of exposure at different temperatures on the antigenicity of the vaccine.

Stability of the mumps CF antigen under conditions of prolonged storage at 5°C. Three samples which had been stored in an electrical refrigerator at approximately 5°C for 3 years were titered. The CF titer of the preparations had been 1:16 when first titered by the same procedure in another laboratory 3 years previously, and the titer was maintained over the 3-year period. It is possible that a decrease in titer might have been masked by differences in technic between the individuals who made the initial and subsequent titrations. The stored vaccine was slightly anticomplementary, but this was evident only in the lowest dilution (1:2) of the antigen.

Stability of the mumps CF antigen at various temperatures. Since vaccines may be subject to various temperatures for varying periods of time before use, an effort was made to determine the effect of different temperatures on the CF titer of the mumps vaccine. Fifty ml of vaccine were pooled and distributed in approximately 10 ml amounts in corked and paraffin-sealed tubes and stored at −70°, −15°, 5°, 22-30° (room temperature) and 37°C for a 7-week period. Antigens were titered in duplicate by the CF test at weekly intervals with the exception of the third week. All samples stood at room temperature for one hour before each titration in order to equalize their temperatures. It was found that the vaccine was quite stable except at −70° and −15°C. The vaccines stored at −15° and at −70°C rapidly lost titer (Table I).

In order to determine more accurately the temperature at which alteration of the CF antigen occurred, a different range of temperatures was employed. Six 10 ml samples were stored at −70°, −23°, −15°, −10°, −5°, and 5°C. Each sample was then titered in duplicate at weekly intervals. Storage at −15°C or lower caused a loss of CF titer to less than 1:2 within one week, while storage at −10°C and −5°C effected a loss to less than 1:2 by the end of the third week. Storage at 5°C had no effect on the titer of the vaccine.

In an effort to discover if the loss of titer in the vaccine which had been stored at subfreezing temperatures was due only to the effects of repeated freezing and thawing, which was necessary in the course of the weekly titrations, 3 ml samples were subjected to the effects of repeated temperature changes which ranged from (a) −15°C to thawing at 5°C, (b) −15°C to thawing at room temperature, and (c) −15°C to thawing at 37°C. The samples were titered in duplicate after having been frozen and thawed 5 times and after repeating the procedure 10 times. None of the various freezing and thawing procedures had any effect upon the titer of the vaccine; it remained 1:32 throughout the experiment.

The loss of ability of the stored vaccine to stimulate the production of CF antibody. It was decided to test the ability of vaccine which had lost its CF titer upon storage at subfreezing temperatures to stimulate the production of CF antibodies in guinea pigs. The use of this vaccine alone failed to elicit any antibody response, so an adjuvant was employed to enhance its antigenicity. Accordingly, 0.4 ml of vaccine the titer of which had dropped from 1:32 to less than 1:2 was mixed with 0.6 ml of Freund's adjuvant(2) and injected subcutaneously into 5 male guinea pigs. Five animals were also injected with 0.4 ml of a similar preparation having a CF titer of 1:32. All animals were bled from the heart prior to injection, the serum collected and stored at −15°C until all samples could be titered on the same day. Blood was again removed from all animals 17 days after injection and all of the sera were titered for the presence of CF antibodies.

From Table II it can be seen that the vac-

TABLE II. Production of Complement Fixing Antibodies in Guinea Pigs upon Injection of Vaccine Which Had Lost Its Complement Fixation Titer upon Storage at −15°C.

Guinea pig	Substance inj.*	Reciprocal of titer at 16 days
1	Adjuvant + vaccine with	256
2	1:32 CF titer	512
3		512
4		512
5		256
	Mean	410
6	Adjuvant + vaccine with	64
7	<1:2 CF titer	64
8		64
9		64
10		128
	Mean	77

* .6 ml adjuvant + .4 ml vaccine.

cine which had lost its CF titer upon storage at —15°C was capable of stimulating the production of CF antibodies in guinea pigs, but such antibody levels were only about 1/5 of those produced by vaccine with a CF titer of 1:32. Essentially the same results were obtained when a vaccine with a titer of 1:8 was used.

Discussion. Our results indicated that the CF antibodies in mumps immune sera were not affected by heating at 60°C for 30 minutes. Thus it appears that the mumps antibody is as stable as the antibody against a number of other viruses. The stability of CF antibody for as long as a week at temperatures of —35°C to +37°C indicated that titers obtained on sterile sera which have been shipped and stored under these conditions would not have changed appreciably since the time the samples were taken.

When the mumps vaccine was stored at 5°C for 3 years it apparently lost no CF titer. It would therefore seem that storage at refrigerator temperature for one year would allow a wide margin of safety.

On storage at freezing temperatures from —5° to —10°C the CF titer and the CF stimulating capacity of the vaccine disappeared or greatly diminished. Thus it seems that storage at freezing temperatures would render a vaccine useless in one week. These results were similar to those of Penttinen(3), Stanley (4), and Salk(5).

The use of an adjuvant with the vaccine in order to obtain detectable or higher levels of CF antibodies is in agreement with the findings of Enders and coworkers(6), Salk(7), and Habel(8).

Summary. 1. The CF antibodies of pooled mumps antisera were stable for 2 months at —15°C, and for 30 minutes at 60°C. 2. Subfreezing temperatures had a markedly deleterious effect on the CF titer of mumps vaccine; temperatures above freezing had little effect; samples stored at 5°C for 3 years showed no drop in titer. After as many as 10 cycles of freezing and thawing, the CF titer of the vaccine remained unchanged. 3. Vaccine which had lost its CF antigen was able to stimulate the production of CF antibodies in guinea pigs provided Freund's adjuvant was used; however, the order of response was approximately 1/5 that produced by the same vaccine with the CF antigens intact. 4. The significance of these observations is discussed in relation to mumps vaccination studies.

1. Habel, K., *Pub. Health Rep.*, 1945, v60, 201.
2. Freund, J., *Ann. Rev. Microbiol.*, 1947, v1, 291.
3. Penttinen, K., *J. Immunol.*, 1950, v64, 165.
4. Stanley, W. M., *J. Exp. Med.*, 1945, v81, 193.
5. Salk, J. E., *J. Immunol.*, 1948, v58, 369.
6. Enders, J. F., Kane, L. W., Cohen, S., and Levens, J. H., *J. Exp. Med.*, 1945, v81, 93.
7. Salk, J. E., and Laurent, A. M., *J. Exp. Med.*, 1952, v95, 429.
8. Habel, K., *Am. J. Hyg.*, 1951, v54, 295.

Received September 30, 1952. P.S.E.B.M., 1952, v81.