a positive one on a later sample.

The result, if any, of the cyclic temperature variation in prolonging the persistence of virus in the fly cannot be evaluated on the basis of the available data. The only thing that can be stated is that under the same conditions of alteration in temperature used with the human strain, murine-adapted strains could not be found beyond the 5th day.

Quantitative virus balance studies were not attempted in these experiments. Without knowledge of the exact amounts of virus taken up by the flies, of the amounts excreted in the first days after the feeding, and the amounts excreted in the 2nd and 3rd weeks following the feeding, it is not possible to answer the important question of whether virus multiplies in the fly. These results however unlike those with the murine-adapted strains, are compatible with the possibility of multiplication.

Summary. Human poliomyelitis virus, as naturally present in stools of poliomyelitic patients has been fed to blow flies, *Phormia regina.* After this feeding, virus was found in the flies for 2 weeks, and in their excreta for 3 weeks.

Results of this type were not encountered when murine-adapted strains of poliomyelitis virus and Theiler's TO strain of spontaneous encephalomyelitis of mice were used. These strains behaved like biologically inert material, such as carmine, in *Phormia regina, Phaenicia sericata* and *Sarcophaga bullata;* following their ingestion they were found in gradually decreasing quantities for a period of 5 days.

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Direct Isolation of Mumps Virus in Chick Embryos.*

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Mumps virus has been adapted to embryonated hen's eggs by the inoculation of relatively large doses of infected monkey parotid by Habel[1] and Levens and Enders[2]. Beveridge, Lind and Anderson[3] using penicillin and sulfonamides as bacteriostatic agents, were able to isolate 4 strains of mumps virus from 12 specimens by direct inoculation of human saliva into the yolk sac of 5 or 6 day embryos. These investigators also isolated one strain by the inoculation of the amniotic sac of 9 or 10 day old embryos. While virus was isolated from 5 of 13 specimens by either yolk sac or amniotic sac inoculation, bacterial contamination was a frequent source of difficulty. Several specimens could not be passed satisfactorily for this reason.

The present report deals with the successful isolation of mumps virus from 8 of 9 patients by the inoculation of saliva into the amniotic sac of 7 or 8 day old chick embryos.

Materials and Methods. Saliva was collected from patients ill with mumps during the acute, inflammatory stage of the disease. This material was emulsified with approximately equal parts of sterile infusion broth. Centrifugation at about 3000 r.p.m. in an angle centrifuge for 15 minutes served to deposit large particles, and presumably, a portion of the bacteria. Penicillin and streptomycin were added to the supernatant to a final concentration of 250 and 2500 units per milliliter, respectively, and the mixture of

* Supported in part by a grant from the Research Grants Division, National Institute of Health, United States Public Health Service.

1 Habel, Karl, *Public Health Rep.,* 1945, **60**, 201.

2 Levens, J., and Enders, J., *Science,* 1945, **102**, 117.

3 Beveridge, W. I. B., Lind, P. E., and Anderson, S. G., *Australian J. Exp. Biol. and M. Sc.,* 1946, **24**, 15.

TABLE I.
Isolation of Mumps Virus Directly from Patients by Amniotic Sac Inoculation of Chick Embryos.

Patient	Age	Day of disease	Virus isolation	First positive embryo passage	Complement fixation test on patient's sera			
					Acute		Conv.	
					Day	Titer	Day	Titer
P.L.	4 yr	3	+	1st	—	—	—	—
S.L.	18 mo	1	+	"	—	—	—	—
A.L.	5½ yr	4	+	2nd	—	—	—	—
E.J.	26 "	2	+	1st	2	<1/4	15	1/256
		5	0	(3 neg.)				
M.W.	8 "	2	0	(3 ")	2	1/64	23	1/64
T.C.	23 "	5	+	2nd	5	<1/8	—	—
E.L.	26 "	1	+	1st	1	<1/8	16	1/64
J.G.	45 "	1	+	"	1	1/8	14	1/8
M.A.	33 "	6	+	"	6	1/16	12	1/256

saliva and antibiotics inoculated without further treatment.

White Leghorn eggs, incubated 8 days at about 38°C., with the air sac uppermost, were routinely used for inoculation. After preliminary candling, a circle 12-15 millimeters in diameter was inscribed over the air sac with a dental drill. This circle of shell was removed with a sterile scalpel. A large drop of sterile broth was then deposited on the shell membrane, and the point of a forceps gently thrust through the drop of fluid and through the shell membrane but not through the underlying chorio-allantoic membrane. The shell membrane was gently removed with forceps. The amniotic sac could then be inoculated under direct vision. One-tenth ml was inoculated into each embryo, and the specimens were incubated at 35-36°C. for 7 days. Usually 10 eggs were inoculated with each specimen.

At the end of incubation, the shell and chorio-allantoic membranes were cut away, and the amniotic fluid removed with a capillary pipette. The fluid was tested for the presence of virus by mixing 0.5 ml with an equal volume of 0.5% pooled, washed chicken erythrocytes, and observing the presence or absence of the characteristic "pattern" of agglutinated cells.[2]

Fluids which gave positive hemagglutination reactions were repassed amniotically. Amniotic membranes of specimens giving negative hemagglutination tests were ground with sterile powdered pyrex glass, mixed with the homologous amniotic fluids, centrifuged briefly, and re-inoculated. Three passages were made before a specimen of saliva was considered negative for mumps virus.

Identification of the infective agent as mumps virus was performed by means of the complement fixation reaction, using acute and convalescent human mumps serum and infected amniotic fluid as antigen.[1] In addition, two specimens were each inoculated in 1 ml amounts into the parotid ducts of two *Macacus rhesus* monkeys. The one monkey of each pair showing the greatest parotid swelling was sacrificed at the period of maximum swelling. The virus was re-isolated in each case from emulsified parotid tissue by amniotic inoculation. The other monkey of each pair was allowed to live for several weeks, and in each instance demonstrated clinical and serological evidence of mumps.

Results. Specimens of saliva from 9 patients with a clinical diagnosis of mumps have been examined. Mumps virus has been isolated from 8 of these patients. In instances in which blood was available for examination, complement fixation tests were performed, using allantoic fluid containing a laboratory strain of mumps virus as antigen.† Data regarding these cases are presented in Table I.

One of these patients, M. W., had typical bilateral mumps 4 years before the illness recently investigated. The more recent illness was mild, characterized by slight fever,

† This strain of virus was kindly supplied by Dr. John Enders, Boston, Mass.

malaise, and unilateral parotitis. Attempted virus isolation gave negative results. Complement fixation tests, although yielding higher values than expected, showed no rise during convalescence, and thus failed to confirm the clinical diagnosis of mumps made by her father, a physician.

The technique described should prove useful in many phases of clinical investigation of mumps, especially for the detection of virus in bodyfluids and secretions at different stages of the disease and in the presence of various complications. Such studies are now in progress.

Summary. A technique for the direct isolation of mumps virus by inoculation of saliva into the amniotic sac of chick embryos is presented in detail. Employing this technique, mumps virus was isolated from 8 of 9 patients with clinical mumps.

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Electroencephalogram of Cats Subjected to Repeated Minimal Convulsive Doses of Electricity.

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In a previous study it was shown that the cat could survive a surprisingly large number of repeated convulsions when these were induced by minimal convulsive doses (MCD) of electricity.[1] In the course of such studies it became of interest to determine what electroencephalographic (EEG) alterations took place, especially since the gross behavioral changes of the animals were not particularly striking. A number of other investigators have already described the EEG in several species of normal animals—*e. g.* cat, dog, rabbit and monkey. Reports concerning the brain wave activity following electrically-induced convulsions, however, are relatively meager[2-6] and no data has so far been made available depicting the EEG changes in animals as their convulsions were continued.

It is, therefore, the purpose of this communication to report such a study in which the progressive changes in the electroencephalogram of cats subjected to frequently repeated convulsions were observed.

For this purpose 12 adult cats were subjected to repeated electrically-induced convulsions at approximately 5 minute intervals with minimal convulsive doses of electricity (alternating current). Solder electrodes were applied 20 mm cephalad to the external occipital protuberance and 10 mm from the midline on each side of the head which corresponded to bilaterally homologous areas of the underlying occipital lobes. These electrodes were held in contact with the shaved scalp previously treated with electrode paste (Sanborn) by the aid of parlodion and were led into the Garceau electroencephalograph. All animals were kept in a shielded box during recordings.

Results. Of the 12 cats employed, 7 succumbed and 5 survived. Because the electroencephalographic patterns were essentially similar in all animals the results obtained can best be represented by the following three plates.

[1] Rubinstein, H. S., and Kurland, A. A., in press.

[2] Lowenbach, H., and Lyman, R. S., *J. Neurol. and Psychiat.*, 1940, **3**, 336.

[3] Bawera, S. E., Lewis, N. D. C., Pacella, B. L., and Kalinowsky, L., *Tr. Am. Neuro. Assn.*, 1942, **68**, 31.

[4] Rheinberger, M. B., and Jasper, H. H., *Am. J. Physiol.*, 1937, **119**, 186.

[5] Clark, S. L., Chambers, W., and Baker, C. F., *Anat. Rec.*, 1942, **82**, 482.

[6] Clark, S. L., and Ward, J. W., *J. Neurophysiol.*, 1945, **8**, 99.