weights of the placenta and fetal membranes, since they are believed to be a primary source of amniotic fluid.

Summary. Meclizine, administered to pregnant Sprague-Dawley rats on the 12th, 13th and 14th days of gestation in a dosage (50 mg per day), which induced palatal clefts in almost one hundred percent of the fetuses, resulted in a significantly elevated volume of amniotic fluid on the 15th day of gestation. Thereafter, progressive decline to a normal value on the 18th gestational day and a significantly depressed amniotic fluid volume on the 19th day of gestation occurred. Fetal weights were approximately the same in experimental and control animals. The weights of the placenta and fetal membranes combined were significantly less than normal on the 16th, 17th, 18th, and 19th days of gestation. Possible mechanisms by which the alterations in amniotic fluid were effected are discussed.

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Experimental Rubella in Rhesus Monkeys.* (28787)

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The role of maternal rubella in the production of congenital malformations of the human fetus has long been recognized(1), and the recent isolation and identification of the rubella agent(2,3) lends importance to the development of an *in vivo* system in which to study this phenomenon. Many years ago Hess reported that inoculation of defibrinated blood from rubella patients failed to produce a rash in 4 rhesus monkeys so tested(4). In 1942, however, Habel described the production of a light, scattered, macular rash with leukopenia in 12 of 41 rhesus monkeys inoculated with nasal washings or blood from patients in the early stages of rubella. Another 15 animals responded with leukopenia, but no exanthem (5). Utilizing similar techniques, Krugman *et al* were unable to induce rubella in 6 cynomologous monkeys(6), while a recent communication by Sigurdardottir *et al* indicates that rubella with rash can be produced experimentally in African green monkeys(7). The possibility that laboratory animals during the various stages of their procurement may contract human diseases and develop immunity thereto is exemplified by the experience with rubeola in rhesus monkeys(8). Since such an occurrence might explain the irregular response of monkeys challenged with the rubella agent, a study was designed to test the thesis that rhesus monkeys recently trapped from a colony isolated from contact with man and his microbial complement, are susceptible to infection with rubella virus.

Materials and methods. Rhesus monkeys. The source of presumed susceptible animals was the isolated colony of rhesus monkeys maintained by the Laboratory of Perinatal Physiology, Nat. Inst. of Neurological Diseases and Blindness, on Cayo Santiago, a

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small island off the southeast coast of Puerto Rico. This colony was established in 1938, and the present population represents the progeny of the original stock. No new animals have been introduced, and human contact has been remote and limited to a small number of investigators observing the colony. Eleven yearling male monkeys were trapped and transported immediately to the facilities of the Laboratory of Perinatal Physiology in San Juan, Puerto Rico, where they were placed in separate cages in an isolated room. Six of these animals were used in the studies described here, while the others were used in a concomitant, but independent rubella experiment conducted by John L. Sever, M.D. of Nat. Inst. of Neurological Diseases and Blindness(9).

Clinical specimens. Through the cooperation of Naval Medical Research Unit No. 4, Great Lakes, Ill., throat washings and serum specimens were obtained from naval recruits in the acute phases of rubella. Specimens from 2 patients were selected for animal inoculations. These materials had been collected 6 hours after onset of the patients' rubelliform exanthem, which was the principal manifestation of an illness otherwise characterized by posterior auricular or occipital lymphadenopathy, peripheral leukocyte counts of 6350 and 7050 per mm^3 respectively, and throat cultures which were negative for beta hemolytic streptococci. Serum from a third subject, a child in the acute stage of rubella, was kindly supplied by Dorothy M. Horstmann, M.D. Convalescent sera were obtained from all patients 3 weeks after collection of the acute specimens and were stored at –20°C. Throat washings and acute sera were quick-frozen promptly after collection and were stored at –70°C. Before use, throat washings were rendered bacteriologically sterile by incubation with 10,000 units of penicillin and 2,000 μg of streptomycin per milliliter for 30 minutes at room temperature.

To rule out the presence of viral agents other than rubella, the throat washings were inoculated by various routes into rabbits, guinea pigs, and adult and suckling mice, and by the intracerebral route into standard laboratory rhesus monkeys. No effect was detected in any of the animals. In addition, inoculation and a single passage of the throat washings in primary rhesus monkey kidney and human amnion cultures failed to produce any cytopathic effects. The techniques described by Parkman, Buescher, and Artenstein for cultivation of rubella virus in African green monkey kidney cell cultures and for detection of neutralizing antibodies to this agent(2) were not available in this laboratory at time of initiation of this study, and subsequent tests in this system of the specimens used for animal inoculation failed to show the presence of rubella virus. However, serologic tests on paired sera from each of the 3 rubella patients from whom these specimens were obtained showed a rise in neutralizing antibody titer of greater than 8-fold, and inoculation of the acute serum from one of the naval recruits (subject NR18) into children at the Willowbrook State School, New York, produced a rubelliform exanthem in 3 of 7 children. In tests on specimens from one of these children, virus was isolated from a throat swab and serum sample collected on the day of appearance of the subject's rash, and from serum specimens collected on the 4th through 6th days preceding appearance of rash. A rise in neutralizing antibody titer to rubella virus from less than 1:2 in the pre-inoculation serum sample to 1:16 in the convalescent specimen was also noted in this subject.†

Animal inoculation procedures. Each animal was examined, and a venous blood sample was obtained just prior to inoculation. Peripheral total leukocyte and differential counts were determined, and the animals' rectal temperatures were recorded. The hair of the neck, anterior chest, abdomen, and inner aspects of the thighs was clipped to facilitate subsequent examinations for rash, and the monkeys were inoculated as indicated in Table I.

Observation of inoculated animals. Each

† The tests on human subjects at the Willowbrook State School were kindly conducted by Drs. M. R. Balsamo, J. P. Giles, R. H. Green, S. Krugman, and G. S. Mirick, Depts. of Pediatrics and Medicine, New York Univ. School of Medicine.

TABLE I. Dose and Route of Inoculation of *M. rhesus* Monkeys with Clinical Specimens from Rubella Patients.

Monkey No.	Material inoculated	Dose and route of inoculation
1 and 2	Acute serum from child with rubella	0.5 ml (diluted to 1.0 ml in physiologic saline) intramuscularly
3	Throat washing from naval recruit NR 17	1.0 ml dropped into nose and nebulized into throat 1.0 ml intraperitoneally
	Acute serum from naval recruit NR 17	2.0 ml intramuscularly
4	Throat washing from naval recruit NR 17	0.5 ml (diluted to 2.0 ml in saline) nebulized into nose and throat 1.5 ml intraperitoneally
	Acute serum from naval recruit NR 17	2.0 ml intramuscularly
5 and 6	Throat washing from naval recruit NR 18	0.5 ml dropped into nose 1.5 ml intraperitoneally
	Acute serum from naval recruit NR 18	2.0 ml intramuscularly

animal was taken from its cage for examination once daily for 21 days following inoculation. Examination was made for: (1) general signs of illness, (2) coryza, (3) enlargement of posterior auricular and occipital lymph nodes, and (4) presence of a rash on the face, neck, anterior chest, abdomen, and inner aspects of the thighs, both by sunlight and artificial light. Rectal temperature was recorded, and a small blood sample was drawn by saphenous venipuncture for leukocyte and differential counts. Convalescent blood specimens were obtained on the 21st and 38th days after inoculation.

Virus isolations and neutralization tests. Neutralization tests and procedures for rubella virus isolation were performed according to the methods described by Parkman, Buescher, and Artenstein(*2*), except that a dose of 100 tissue culture interfering $doses_{50}$ ($TCInD_{50}$) of rubella virus was used in the neutralization tests instead of 1-10 $TCInD_{50}$ as recommended by these authors. The sera tested were not inactivated. Seed rubella virus was kindly supplied by Paul D. Parkman, M.D.

Results. During the 21 days of observation following inoculation, none of the monkeys developed any physical signs of illness, including exanthems, except Monkey #6 which had mild diarrhea for 3 days (4th through 6th days after inoculation). A rectal swab culture from this animal was negative for Shigella, Salmonella, and pathogenic coliform bacteria, and the diarrhea ceased after symptomatic treatment. Rubella virus was not detectable in serum specimens obtained from the animals at various times between the 6th and 13th days after inoculation. However, in 4 of the 6 animals, those inoculated with throat washings and acute sera from the 2 naval recruits, significant rises in neutralizing antibody titer to rubella virus were demonstrated (Table II). No such rise was seen in the 2 animals inoculated only with acute serum from the child with rubella, and none of the animals showed neutralizing antibody in pre-inoculation serum specimens.

Records of daily rectal temperatures and serial total leukocyte and differential counts were characterized by considerable day to day variability. Plots of rectal temperatures

TABLE II. Neutralizing Antibody Titers to Rubella Virus in *M. rhesus* Monkeys Inoculated with Clinical Specimens from Rubella Patients.

	Monkey No.					
	1	2	3	4	5	6
Pre-inoculation titer	<1:2	<1:2	<1:2	<1:2	<1:2	<1:2
3-wk post inoculation titer	<1:2	<1:2	1:16	1:32	1:16	1:16
5-wk post inoculation titer	<1:2	<1:2	1:32	1:32	1:32	1:16

showed no consistent trends in any of the animals, and while records of leukocyte and differential counts were suggestive of a gradual development of leukopenia and relative lymphocytosis in 3 of the 4 animals showing rises in rubella neutralizing antibody titer, similar, although less marked changes were noted in the 2 animals showing no serologic evidence of rubella infection.

Discussion. Although none of the animals studied showed overt manifestations of rubella infection, the serologic data indicate that the animals inoculated with the specimens from the 2 naval recruits experienced at least a specific antigenic response to these materials, and probably sustained an inapparent infection. While the rubella virus was not detected by tissue culture methods(2) in the specimens used for monkey inoculation, its presence in one of the acute sera (from naval recruit NR18) with which 2 of the animals were injected was demonstrated by production of a rubelliform illness following its injection into humans. Serum from the child in the acute phase of rubella apparently contained insufficient virus to stimulate antibody production in the animals tested. It is inferred, therefore, that the quantity of active virus contained in the clinical specimens was small, and it seems unlikely that an antibody response of the degree seen in 4 of the 6 inoculated monkeys could have occurred in the absence of active infection. However, the possibility that this was a serologic reaction to a larger amount of inactive, and therefore undetected viral antigen cannot be excluded completely.

The serologic data show that the animals used in this study had no demonstrable neutralizing antibody to rubella virus before challenge, and thus had no immunity which could be explained on the basis of prior incidental exposure to rubella. This is in contrast to the report by Sever *et al* that 2 of the 5 monkeys, captured from this colony at the same time as the animals used in this study, had demonstrable neutralizing antibody to rubella virus in pre-inoculation serum specimens(9). This difference in findings is attributable perhaps to the small number of animals tested in the 2 studies or to the different neutralization test techniques utilized.

Tabulations of body temperature and leukocyte responses of the monkeys inoculated in this study showed these parameters to be subject to appreciable daily fluctuations, and therefore of very limited reliability as indices of infection. The variations previously reported in the response of rhesus monkeys to the rubella agent(4,5), are probably attributable to individual variations in the animals or to differences in the materials inoculated, and the situation is perhaps analogous to that in man in which rubella infection may occur with or without an exanthem(5,6). It is concluded that rhesus monkeys are probably susceptible to infection with rubella virus, but that the infection is usually of the inapparent type, best detected at present by serologic methods.

Summary. In an attempt to transmit rubella to rhesus monkeys, 6 yearling male animals from an isolated, and therefore presumably susceptible population, were inoculated by several routes with throat washings and acute phase sera from selected rubella patients. During 21 days of observation following inoculation, none of the animals showed any physical signs of rubella. Serial daily total and differential leukocyte counts showed leukopenia and lymphocytosis of slight degree. In none of the animals tested was neutralizing antibody to rubella virus demonstrable prior to inoculation, but neutralization tests performed on pre-inoculation and post-inoculation sera showed a significant rise in titer in 4 of the 6 animals inoculated. It is concluded, therefore, that rhesus monkeys are probably susceptible to infection with rubella virus, but that the infection is usually of the inapparent type.

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Immunochemical Studies with Ovine Prolactin. (28788)

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Pierce and Carsten, using a starch gel electrodialysis technique, demonstrated that there are 3 components in highly purified ovine prolactin (N.I.H.-PS-3)(1). Cole and Li(2), using zone electrophoresis on starch gel also obtained 3 components, each of which possessed biological activity according to the standard pigeon crop sac assay method of Riddle, Bates and Dykshorn(3,4). By a similar technique Ferguson and Wallace observed 4 components with crop sac activity (5). Reisfeld *et al*(6) isolated 3 components from ovine prolactin by column chromatography on diethylaminoethyl cellulose (DEAE-C) and noted that component I, which had the greatest biological activity of the 3, also possessed a different C-terminal amino acid group from components II and III. Squire, Starman and Li(7) have studied the sedimentation velocity of ovine prolactin over a wide pH range and observed that despite the high degree of purity, the hormone is polydisperse, having a "multiplicity of biologically active components."

The inhomogeneity of purified ovine prolactin, as demonstrated by these physical-chemical methods has also been supported by recent immuno-chemical studies by Emmart, Bates and Spicer(8), who observed that ovine prolactin (N.I.H.-PS-3), when diluted serially and precipitated by a constant volume of antisera, exhibited 3 zones of precipitation, one zone of higher intensity and 2 of lesser magnitude. Concurrently multiple precipitin bands were observed in Ouchterlony plates. Since these results were in contrast to the single precipitin band in agar gel reported by Hayashida(9), these immuno-chemical studies have been extended to include several preparations of active ovine prolactin, and fractions of prolactin isolated by countercurrent distribution, by chromatography on DEAE cellulose column and by filtration through Sephadex. The following results indicate that fractions prepared by these methods differ in degree of biological activity, in rate of diffusion in agar gel, as well as in immunochemical response to antibody. Furthermore, since antibody to prolactin is capable of precipitating both active prolactin as well as prolactin fractions which have lost their biological activity, the precipitin reaction appears not to be related directly to the biological activity of prolactin.

Materials and methods. Ovine prolactin (N.I.H.-PS-3) with a potency of 15 I.U. per mg was obtained from the Endocrinology Study Section of Nat. Inst. of Health. This lot of purified prolactin was consistently used for immunization in rabbits as previously described(8). Two other ovine prolactin preparations, designated as S-89 and 71713, pre-