Live Attenuated Mumps Virus Vaccine. 1. Vaccine Development. (31599)

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Mumps is usually a minor infectious disease of childhood. In a portion of cases, however, the clinical consequences of systemic infection with mumps virus may be quite severe and this is especially true when the illness is delayed until adulthood. The personal discomfort and time loss from productive effort resulting from ordinary mumps and the serious or even fatal aspects of the extraordinary case appear to justify development and application of an effective live virus vaccine.

During the past several years, this laboratory has been engaged in efforts to develop an attenuated live mumps virus vaccine which would produce a noncontagious infection in man, would fail to cause significant clinical reaction, would induce protective antibody in essentially all recipients, and would afford solid protection against mumps upon exposure. Studies of a number of candidate mumps viruses led to the development of the Jeryl Lynn (B level) vaccine strain which has fulfilled all these criteria. As recorded elsewhere (2,3), this vaccine has now been tested in more than 400 susceptible children in controlled field studies and has proved to be highly effective both in stimulating antibody and in affording protection against challenge with mumps on exposure in nature.

The present report describes the development of the vaccine. Other reports (1-4) present data relative to the early clinical and large-scale field studies of the vaccine.

Materials and methods. Viral strains. Female patient (J.L.H.) developed clinical mumps with unilateral parotitis on March 30, 1963. There were no clinical complications. Virus strain Jeryl Lynn was isolated by amniotic inoculation into embryonated hens' eggs from a chicken flock which was free of avian leukosis and other detectable infections. The virus was passed further in embryonated hens' eggs and in cell cultures of chick embryo. The ABC line of mumps virus was initiated by passage in HeLa cell cultures of throat washings from a patient with clinical mumps. The virus was obtained from Dr. Werner Henle in HeLa cell passage 7 and was passed 3 additional times in HeLa cell cultures in this laboratory. This agent was employed as "virulent" virus control. The Barnes (B82) strain of mumps virus used for the hemagglutination-inhibition tests was received from Dr. Klaus Hummeler and was passed in this laboratory in embryonated hens' eggs *via* the allantoic route. Early passage of Jeryl Lynn strain mumps and a Farina strain of mumps virus isolated in this laboratory were also used.

Cell cultures. Primary cell cultures of chick embryo were prepared by the ordinary trypsinization procedure using 9- to 11-day-old decerebrated chick embryos from a leukosis-free chicken flock. Cell propagation was in medium 199 containing 2% inactivated calf serum and cell culture maintenance for virus test purpose was in the same medium. Primary cell cultures of grivet monkey kidney, rhesus monkey kidney, and human amnion and cell cultures of the HeLa and WISH stable amnion lines were prepared and maintained by ordinary procedures.

Titrations for viral infectivity were carried out employing serial 10-fold dilutions of virus in primary cell cultures of grivet monkey kidney or chick embryo or in HeLa cells. Ten culture tubes were ordinarily used per dilution and the inoculum dose was 0.1 ml per tube. The cultures were incubated stationary at 36°C and were read on the seventh day of incubation for cytopathic change and for capacity to hemadsorb guinea pig red blood cells. Hemadsorption was found to improve the sensitivity and specificity of the test. The 50% tissue culture infectious dose was calculated according to Karber(5). *Hemagglutination-inhibition (HI)* tests were carried

TABLE I. Serologic Responses Against Mumps Virus Among Initially Seronegative Children Who Were Given Lot 136 or 137 of Live Attenuated Jeryl Lynn Strain Mumps Virus Vaccine.

Lot 137 vaccine (B level)			Lot 136 vaccine (A level)		
Case No.	Neut. titer	HI titer	Case No.	Neut. titer	HI titer
140	32	40	121	128	40
123	16	40	99	128	160
133	16	20	112	128	80
107	16	10	134	32	20
106	16	10	136	32	40
115	16	10	130	16	40
129	16	20	98	16	20
114	16	10	117	16	20
109	8	10	126	16	10
122	8	10	138	16	20
124	8	10	142	16	10
120	8	5	95	8	10
102	4	10	97	8	5
119	4	5	132	8	10
			105	4	5
			110	4	5
G.M.*	11	12	G.M.	19	18

Mumps antibody measured 4 wk after vaccination.
* G.M. = Geometric mean titer.

out by usual procedures employing as antigen allantoic fluid from embryonated hens' eggs infected with the Barnes (B82) strain of mumps virus and using washed chicken erythrocytes as indicator. All sera were inactivated at 56°C for 30 minutes prior to test and were absorbed with kaolin and chick erythrocytes to remove nonspecific inhibitors of hemagglutination. The titer was the highest initial dilution of serum which caused total suppression of hemagglutination in the tests. *Serum neutralization* tests were performed in chick embryo cell cultures employing the Jeryl Lynn strain virus which had been passed a total of 7 times in embryonated hens' eggs and in chick embryo cell culture. The sera were inactivated at 56°C for 30 minutes prior to test and 30 to 100 $TCID_{50}$ of virus were employed as test dose. The tests were read for hemadsorption on the fifth day following incubation at 32°C and the titer was the highest initial dilution of serum which showed total absence of hemadsorption.

Results. Vaccine preparation. Two vaccines were prepared from the Jeryl Lynn strain of mumps virus. Lot 136 was prepared from virus at twelfth passage in chick embryo and chick embryo cell culture, combined, and designated level A. This virus retained the capacity to produce mild parotitis in a small portion of children who received it(1). Lot 137 was prepared from virus at the seventeenth passage in these systems and was designated level B. The latter lot was used in all the mass field trials(2-4) of the vaccine and passage level B was elected for routine vaccine preparation. The virus for vaccine was propagated in cell cultures of chick embryo maintained in a serum-free medium. The virus-containing fluids were stored frozen at −65°C in an electric deepfreeze during the conduct of the essential preliminary tests. A suitable stabilizer was added to the vaccine after clarification and prior to filling and drying. Laboratory testing of the vaccine, with appropriate modification, was generally consistent with the regulations for live measles virus vaccine specified in the Standards of the U. S. Public Health Service(6). Included were tests for safety in mice, embryonated hens' eggs and cortisonized monkeys and in cell cultures of grivet monkey kidney, rhesus monkey kidney, chick embryo, rabbit kidney and the HeLa line. Appropriate tests for microbial sterility including PPLO and for freedom from *Mycobacterium tuberculosis* and avian leukosis virus were carried out. The final vaccine was titrated for infectivity in GMK cell culture and identity was established in serum neutralization tests with standard mumps antiserum prepared in horses. Two lots of Jeryl Lynn strain mumps vaccine were employed in the present study. Lot 136 prepared from level A virus had an infectivity titer of $10^{-3.0}$/0.1 ml as measured in grivet monkey kidney using the hemadsorption endpoint. Lot 137 was prepared from level B virus and titered $10^{-3.5}$ in the same test.

The succeeding sections of the present report describe important observations relative to these 2 lots of vaccine.

Tests in children. The detailed serological, virus isolation, and clinical findings among institutionalized children who were given these vaccines have been recorded by Stokes *et al* (1). The 28-day serologic responses among a portion of these children are presented in Table I for illustrative purpose. All recipi-

TABLE II. Antibody Responses to Jeryl Lynn Strain Live Mumps Virus Vaccines in Susceptible Cortisonized Rhesus Monkeys Injected by the Combined Thalamic, Spinal and Muscular Routes.

Reciprocal of antibody titer following vaccine*					
Lot 137 vaccine (level B)			Lot 136 vaccine (level A)		
Monkey No.	Neutralization	HI	Monkey No.	Neutralization	HI
Inoculated					
8	16	5	8	128	80
6	8	<5	9	128	160
7	8	<5	10	32	40
1	4	<5	1	16	10
9	4	<5	3	16	10
10	4	<5	7	16	20
13	2	5	4	4	5
4	2	<5	6	4	<5
14	<2	<5	2	2	<5
3	<2	<5	5	<1	<5
Geometric mean:	3	2		11	9
Uninoculated controls†					
11	<2	<5	11	<1	<5
12	<2	<5	12	<1	<5

* Bled on day 21 following vaccine.
† Held in same room as contacts.

ents of both vaccines responded serologically with the development of both neutralizing and HI antibody. The geometric mean neutralizing and HI titers for lot 137 vaccine were 1:11 and 1:12, respectively. The mean titers following lot 136 vaccine were somewhat higher, *viz.*, 1:19 and 1:18. It was noted(1) that a few of the children who received lot 136 (level A) vaccine developed parotitis and excreted mumps virus at some time during their infection. The recipients of lot 137 (level B) vaccine did neither. The serologic and clinical findings indicated that level A vaccine was less attenuated than level B.

Tests in monkeys. Tests were carried out in rhesus monkeys which were initially seronegative for mumps using 10 monkeys for each vaccine lot. Each monkey was given 200 mg of cortisone acetate intramuscularly and 0.5 ml of undiluted vaccine distributed bilaterally into the thalamus, 0.5 ml into the lumbar cord, and 1.0 ml intramuscularly. A few uninoculated monkeys were held as controls. The animals were observed for 21 days for development of clinical signs and at the end of this period were bled, sacrificed and perfused with dilute formalin. Gross pathologic examination was made of both test and control monkeys and samples of brain, cord, testis, epididymis, ovary, pancreas, and parotid glands of each monkey were taken for histologic examination.*

The serologic findings in the tests in monkeys are given in Table II. All but 2 monkeys which received lot 137 and all but 1 of those which were given lot 136 developed neutralizing antibody. Only 2 monkeys given lot 137 vaccine developed HI antibody, whereas 7 of the 10 given lot 136 responded in this way. It is clear that the seroconversion rate and the mean antibody levels achieved following lot 136 were greater than for lot 137 vaccine. The greater serologic response to lot 136 compared with lot 137 was also seen in the children and the findings in general were consistent with a lesser degree of attenuation of A level lot 136 compared with B level lot 137 vaccine.

No lesions were noted on gross pathologic examination. The histopathologic findings in the tissues of the monkeys were not remarkable and there was no significant difference in the results obtained with lot 136 compared with lot 137. Very slight cellular infiltration or vascular cuffing in the choroid plexus and in the walls of the ventricular system possibly related to mumps virus infection was noted in the majority of animals. No significant pathologic lesions referable to the vaccine was observed in any of the remaining tissues which were examined. For control purpose, the ABC "virulent" and early egg passage of the Jeryl Lynn strain (passage 7) and a Farina strain (passage 5) were all tested in monkeys. The changes were not significantly different qualitatively or quantitatively from those described above for Jeryl Lynn lots 136 and 137 vaccine.

Marker tests for virulence. Lots 136 and 137 of live attenuated Jeryl Lynn strain mumps virus were compared with "virulent" ABC strain in infectivity titrations carried out in a variety of primary and line cell cultures and the findings are given in Table III. There was no remarkable difference be-

* Monkey testing and gross pathologic examination was carried out by Dr. R. Hoey. Histopathologic examination and interpretations were made by Dr. H. Peck.

TABLE III. Comparison of Jeryl Lynn Strain Live Attenuated Mumps Virus Strains with "Virulent" Mumps Virus.

Virus titrations: Cell cultures used to test	Virus titrations: Methods for detecting virus presence	Neg. log_{10} infectivity in titrations of Attenuated virus: Lot 137 (level B)	Attenuated virus: Lot 136 (level A)	"Virulent" virus: ABC strain (control)
Primary grivet kidney	CPE	2.5	2.8	4.0
	Hemadsorpt.	3.5	3.0	4.0
Primary rhesus kidney	CPE	3.0	3.3	4.3
	Hemadsorpt.	3.0	3.3	4.3
Primary human amnion	CPE	<0	<0	3.8
	Hemadsorpt.	2.0	1.8	3.8
Stable human amnion (WISH)	CPE	3.3	3.0	4.0
	Hemadsorpt.	2.8	2.5	4.3
HeLa (stable human malignant)	CPE	3.3	3.3	4.0
	Hemadsorpt.	3.0	2.8	4.0
Chick embryo	CPE	4.3	4.3	2.8
	Hemadsorpt.	4.6	≥4.8	3.8

tween the "virulent" and attenuated viruses for any of the cell culture systems used except for primary human amnion. Failure of the attenuated strains to show cytopathic effect in primary human amnion cells and the reduced infectivity titer noted on hemadsorption appeared to distinguish clearly between the "virulent" and attenuated mumps viruses but did not distinguish B level lot 137 attenuated virus from A level lot 136. A minor differentiative quality between the "virulent" and attenuated strains was noted in the lesser capability of "virulent" ABC virus to destroy chick embryo cells compared with the chick embryo adapted Jeryl Lynn strain.

The cytopathic effect caused by attenuated Jeryl Lynn strain mumps virus in chick embryo cell cultures and shown in Fig. 1 consisted mainly of cell separation on the sheet followed by cell lysis, clumping, and total or near total destruction of essentially all cells by the seventh day following infection. Syncytium formation was usually present in the early stages of degeneration.

Stability on storage of Jeryl Lynn strain live mumps virus vaccine. Dried B level lot 137 vaccine and rehydrated vaccine of the same lot were stored at various temperatures for the time periods shown in Table IV. The dried vaccine appeared to be fully stable on storage at 26°C for about 2 weeks. Significant drop in titer appeared to occur between the first and third days at 36°C and within 2 hours at 56°C. The rehydrated virus could be stored at 4°C for at least 8 hours without significant diminution of titer. There appeared to be a diminution of titer by 24 hours at 4°C, by 4 hours at 26°C and by 2 hours at 36°C.

Additional data on storage stability of dried vaccine were obtained in tests of an attenuated Farina strain mumps virus which was at the twelfth chick embryo-chick embryo cell culture passage level and otherwise comparable to Jeryl Lynn strain vaccines. The Farina vaccine was prepared in the same way as Jeryl Lynn vaccine lot 137. The find-

TABLE IV. Stability on Storage at Various Temperatures of Lot 137 Jeryl Lynn Strain (Level B) Live Mumps Virus Vaccine as Dried Material and After Rehydration.

Length of storage period	Neg. log_{10} infectivity titer: Dried vaccine, storage temp. 26°C	Dried 36°C	Dried 56°C	Rehydrated vaccine, storage temp. 4°C	Rehydrated 26°C	Rehydrated 36°C
0	3.9	3.9	3.9	4.3	4.3	4.3
1 hr	N.D.*	N.D.	*N.D.*	3.8	3.8	*4.0*
2 "	"	"	3.0	4.0	*3.8*	3.3
4 "	"	"	3.1	3.8	3.3	3.0
6 "	"	"	2.8	3.8	2.8	2.8
8 "	"	"	1.8	*4.0*	3.5	2.8
1 day	"	*4.3*	<0	3.3	2.0	1.0
3 days	"	3.3	N.D.			
7 "	3.8	3.1	"			
9 "	N.D.	2.9	"			
14 "	*3.6*	1.9	"			
28 "	2.7	N.D.	"			
42 "	2.5	"				

* N.D. = Not done.

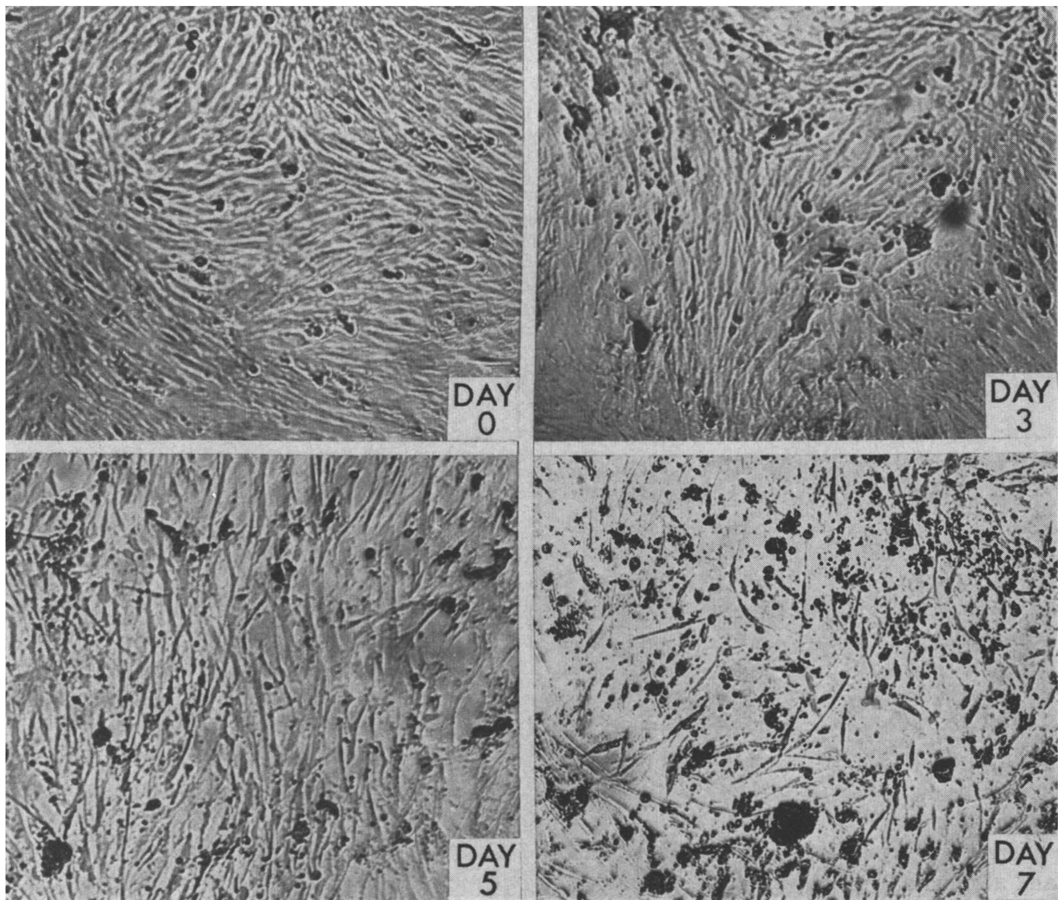

FIG. 1. Photos showing progression of cytopathic changes in cell cultures of chick embryo infected with Jeryl Lynn (B level) vaccine strain of mumps virus. 70 × magnification.

ings shown in Table V demonstrate that the dried vaccine was stable for at least 10 months when stored at 4°C but lost appreciable infectivity after more than 2 weeks' exposure to 25°C or after 3 days' exposure to 37°C. The virus was quite stable when stored as frozen wet material at −70°C.

Mumps antibody content of human immune globulin. Sixteen lots of commercial human immune globulin purchased at random were titrated for content of neutralizing antibody against the Jeryl Lynn strain of mumps virus. The results are given in Table VI. There was remarkable variation in mumps antibody titer from lot to lot ranging from 1:2048 to 1:64. The median titer was 1:256.

Discussion. Mumps is generally regarded as an acute infection in which the usual presenting sign is parotitis but in which severe and even lethal result may occur, especially when first infection occurs in adulthood(7-10). The infection is generalized and there

TABLE V. Stability on Storage at Various Temperatures of Dried Live Mumps Virus Vaccine Farina M-2226-101.

	Neg. $\log_{10}$ of infectivity titer			
Length of storage	Frozen virus control (−70°C)	Storage temp. of dried vaccine 37°C	25°C	4°C
0	2.3	2.3	2.3	2.3
1 day	N.D.*	*2.0*	2.3	N.D.
3 days	N.D.	1.5	2.3	N.D.
7 "	2.0	1.3	1.8	2.3
14 "	2.0	1.3	*1.8*	2.0
1 mo	1.8	<0	1.0	2.0
2 "	2.0	<0	1.0	2.0
3 "	2.0	<0	0.5	2.0
6.5 "	2.3	N.D.	<0	2.0
10 "	2.5	N.D.	N.D.	2.3

* N.D. = Not done.

TABLE VI. Neutralizing Antibody Against Mumps Virus in Lots of Human Immune Globulin.

Manufacturer	Lot	Reciprocal of titer
1	A—X9	2048
	B—X8	64
2	C—43	256
	D—70	1024
3	E—90	1024
	F—26	1024
4	G—15B	512
	H—52A	256
	I—30A	256
	J—50B	128
5	K—69B	256
	L—89B	256
	M—77B	128
	N—72B	128
6	O—05	2048
	P—95	512

may be involvement of the nervous system, testis, epididymis, prostate, ovary, mammary glands, vulvovaginal glands, thymus, pancreas, liver, spleen, kidneys, thyroid gland, eye and ear as well as the parotids. Most serious involvement may be in the nervous system(11) with production of meningoencephalitis which is lethal on rare occasion, transverse myelitis, ascending paralysis, hemiplegia, optic neuritis, and unilateral or bilateral eighth nerve deafness which may be permanent. The orchitis, which occurs especially in adolescents or adults, frequently leads to testicular atrophy with impaired fertility or sterility. Severe pancreatitis with subsequent diabetes has been noted(12). Transient arthritis and occasional thrombocytopenic purpura may also occur and the possibility of congenital defect (11,13) such as endocardial fibroelastosis has been considered. These aspects of mumps virus infection as well as the discomfort to the patient and time loss from gainful effort appear to justify application of a safe and effective live mumps virus vaccine.

The B level Jeryl Lynn strain live attenuated mumps virus vaccine described here appears to present the esssential qualifications for an acceptable vaccine for preventing mumps virus infection. The vaccine was prepared in chick embryo cell culture and was tested for safety in a manner consistent with the guidelines established for live attenuated measles virus vaccine(6). As shown in this and other reports(1-4), a single dose of the vaccine induced antibody in approximately 98% of more than 400 children tested who were initially without mumps antibody. The vaccine did not cause fever or other clinical manifestation, including parotitis, in any of the vaccinated children which was in excess of that observed in susceptible contact controls who had not been vaccinated. The virus was not secreted from vaccinated children and was not contagious to susceptible contacts. Mumps antibody induced by the vaccine was retained for at least 7 months in all of a sample of children tested to date, and protective efficacy against natural mumps was shown to last for at least 8 month, the longest period of observation.

Further, the Jeryl Lynn B level vaccine showed a 97% protective efficacy (attack rates: vaccinated 2/100 and control 61/100) against laboratory proved cases of natural mumps in 200 of the children who were at risk to mumps in a controlled study which included 1394 children and which was carried out during 1965-66 in a suburb of Philadelphia(2-4).

As shown in this report, the infectivity of the dried vaccine virus was stable on storage at 4°C and was sufficiently stable at higher temperature and following rehydration to render it practical for routine use.

The tests for safety of the vaccine included inoculation into rhesus monkey *via* the CNS and muscular routes. These animals did not become detectably ill and the lesions which were noted on histopathologic examination of CNS tissue and which were possibly related to mumps virus infection were minimal. Further, the histopathologic findings did not distinguish the B level vaccine from the A level Jeryl Lynn virus which still caused parotitis in a portion of the children. Animals tested in the same manner with "virulent" ABC strain mumps virus, not reported herein, also showed only minimal lesions in the CNS. The A level Jeryl Lynn virus could be distinguished from B level virus only in the induction by the former of a much greater antibody response in monkeys.

Both A and B level Jeryl Lynn strain mumps virus could be distinguished from "virulent" ABC strain based on lack of cytopathic effect and lesser capability of the former to grow in primary human amnion. By contrast, the A and B level Jeryl Lynn virus propagated more readily in cell cultures of chick embryo than did the virulent strain. The tests did not differentiate A level from B level Jeryl Lynn virus and all the viruses including ABC grew well and caused cytopathic effect in primary rhesus and grivet renal cell cultures and in the HeLa and human amnion (WISH) stable line cells. It was apparent, therefore, that there were few markers for attenuation but that the "virulent" virus could be distinguished from the attenuated strains by test in primary human amnion and chick embryo cell cultures. Furthermore, the less attenuated A level virus induced a much greater serologic response in monkeys than did the B level virus chosen for routine vaccine use.

Neutralizing antibody against Jeryl Lynn strain mumps virus was present in all lots of human immune globulin tested. The reason for the considerable variation in titer from lot to lot was not apparent.

Killed mumps virus vaccines have not found routine use in children, apparently because of the lack of producing a durable protective effect(14). Early studies with live virus(15,16) showed that the mumps agent was rapidly attenuated for man by passage in embryonated hens' eggs and that administration of the virus by oral spray was capable of eliciting antibody and a degree of protection without causing typical mumps. It was apparent, however, that the virus became rapidly overattenuated for man(17) and that a narrow range in passage level might be required for a safe and effective vaccine. Smorodintsev and his co-workers(18,19) conducted extensive investigations of live mumps virus vaccines for several years in the U.S.S.R. employing virus prepared originally in embryonated hens' eggs and most recently in chick embryo cell cultures. The virus was given by the intradermal or subcutaneous route. Though the antibody responses appeared to be less than maximal, the vaccines nevertheless afforded an apparent protective efficacy of 90% or greater and the immunity lasted for at least 5 years.

Summary. The development and testing of an attenuated live mumps virus vaccine designated Jeryl Lynn strain, level B, is described. The virus was attenuated by passage in embryonated hens' eggs and in cell cultures of chick embryo and the vaccine was tested for safety and potency by procedures consistent with contemporary standards for live measles virus vaccine. The vaccine was used as a dried product and was sufficiently stable both in dried form and on rehydration to permit use in routine practice. It was possible to distinguish the attenuated virus from a "virulent" mumps virus strain based on cytopathic effect and on titer obtained in tests in primary human amnion and in chick embryo cell cultures but not in cultures of monkey kidney or HeLa or stable human amnion (WISH). It was not possible to distinguish the level B Jeryl Lynn virus from less attenuated level A virus in cell culture but the level B virus did induce considerably less antibody in monkeys than did level A. Both the level A and level B vaccines were highly immunogenic for man. A review was made of additional data published elsewhere in which it was shown that the B level vaccine was highly efficacious in preventing natural mumps and that the virus was neither excreted from vaccinated persons nor was it contagious to susceptible contacts. All of 10 lots of human immune globulin of diverse source tested contained mumps neutralizing antibody in the range of titer of 1:64 to 1:2048 with a median of 1:256.

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Local Ovarian Effects of an Intrauterine Device in Intact and Unilaterally Ovariectomized Guinea Pigs.* (31600)

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Intrauterine foreign bodies produce shortened estrous cycles in cattle, guinea pigs and sheep. In cattle(1) and in sheep(2) a plastic coil in one uterine horn results in shorter estrous cycles when the coil is in the horn adjacent to the ovary containing the corpus luteum (CL) than when the coil is opposite to the CL. The present experiments were designed to study the local utero-ovarian influences of an intrauterine device (IUD) on the CL and on the ovulation rate in guinea pigs.

Methods. A total of 107 virgin guinea pigs weighing 400 to 600 g was used in 3 separate experiments. The animals were examined approximately every 12 hours and the estrous cycle length was determined by means of the vaginal smear technique. The day of estrus was designated day 1 of the estrous cycle. In all 3 studies the IUD consisted of a 7.0 mm length of 3.3 mm diameter plastic tubing.‡ For surgery the guinea pigs were anesthetized with an intraperitoneal injection of pentobarbital sodium, supplemented when necessary with the application of ether. The ovaries and uterine horns were exposed by means of 2 high lumbar, transverse abdominal incisions, one on each side of the body. The IUD was inserted through a small longitudinal incision in the caudal portion of the uterine horn and was pushed forward with a probe to a point approximately 10 mm from the cranial end of the horn. The device was sutured in place and the uterine incision was closed with 5-0 silk suture. The side of insertion of the IUD (right or left horn) was assigned alternately for successive guinea pigs within each treatment group at the time of surgery.

Experiment 1 was conducted during June and July, 1965. It was designed to determine: a) if an IUD, inserted in one uterine

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