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Successful Immunization of Primates with Rabies Vaccine Prepared in Human Diploid Cell Strain WI-38.* (30048)

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At present, anti-rabies vaccines used for the immunization of man are produced in either animal brain or in duck embryos and are inactivated by phenol or by betapropiolactone (BPL). Seven to 21 doses of the vaccines have to be given daily, depending on the severity of exposure.

When sensitization occurs during treatment, the vaccine produced in brain tissue can cause postvaccinal neuroparalytic accidents, and the duck embryo vaccine, not completely free from encephalitogenic properties(1,2) has been known to cause on occasion a severe anaphylactic reaction(3).

The Flury HEP strain of rabies grown in chick embryo(4) has been effectively used as a live virus vaccine for booster inoculation of persons who had previously received inactivated vaccine(5). This live virus vaccine had little antigenic value, though, when used for primary immunization of man and monkeys(4,6,7) as compared with the excellent results obtained in other animal species such as dogs and cattle(8,9,10,11,12).

Thus, an urgent need exists for a highly immunogenic anti-rabies vaccine which can be used safely and effectively at low dosages for treatment of humans after exposure as well as for primary immunization of "high risk" groups.

A preliminary investigation(13) showed that the HEP-Flury strain adapted for growth in human diploid cell strains WI-38 (HDCS) had greater antigenic properties than the same strain grown in embryonated eggs. Also, vaccine prepared from Pitman-Moore (PM) and the challenge virus standard (CVS) strains in HDCS and inactivated by BPL was found to be more antigenic than phenolized vaccine in a mouse potency test (13).

These results warranted further investigation of the 2 types of vaccines produced in HDCS which are designed for immunization of man; therefore, an evaluation of their antigenic potency in primates was planned.

Materials and methods. Animals. Seventy Rhesus monkeys *(Macaca mulatta)* which had been maintained in captivity for a number of years at the National Primate Center, University of California, Davis, were used in these experiments. During the last 2 years, these animals had been exposed to repeated intratracheal instillation of DMBA (7,12-dimethyl-(a)-benzanthracene) in an attempt to ascertain whether it had a carcinogenic effect. The results of this experiment were negative.

As shown in Table I, 42 male and 27 female monkeys, weighing from 3.1 to 5.8 kg (average weight 4.0 kg) formed the experimental group. Forty-two of the animals were previously splenectomized when used in an experimental study of malaria.

As shown in Table I, 7 experimental groups were formed each containing 10 animals randomly selected as to sex and weight. Half of the animals were kept inside the animal building and the other half were kept outside in a shaded pen.

All animals were bled before immunization and on the 7th, 14th, 28th, 56th, and 135th day following the first inoculation of vaccine. After the blood was allowed to coagulate at room temperature, the serum was separated

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TABLE I. Distribution of Monkeys in Experimental Groups.

Exp group	Vaccination: No. of doses	Vaccination: Days of inoculation	♂/♀ ratio	Wt, kg avg and range	Splenecto-mized/Total
HEP/HDCS*	7	1, 3, 5, 7, 9, 11, 13	8/2	3.9 (3.2-4.5)	4/10
	3	1, 6, 11	5/5	4.2 (3.4-5.5)	6/10
	1	1	6/4	4.5 (3.2-5.8)	6/10
PM/BPL	7	1, 3, 5, 7, 9, 11, 13	8/2	4.0 (3.2-5.6)	7/10
	3	1, 6, 11	6/4	4.0 (3.1-5.6)	6/10
HEP/EE	3	1, 6, 11	3/7	4.0 (3.5-4.8)	6/10
DE	7	1, 3, 5, 7, 9, 11, 13	7/3	4.0 (3.3-5.8)	7/10
Total	—	—	43/27	4.0 (3.1-5.8)	42/70

* See *Materials and methods.*

by centrifugation and kept at —20°C for study.

Random bred Swiss white (Webster) mice, 4 to 5 weeks old, of both sexes, were used for serum neutralization tests.

Vaccines. Four types of vaccines were used (Table I):

A live virus tissue culture vaccine—Flury-HEP strain of rabies virus(4) grown in HDCS WI-38 for 49 passages (HEP/HDCS)(13).

An inactivated tissue culture vaccine—Pitman-Moore strain of rabies virus grown in HDCS WI-38 for 38 passages and inactivated by BPL (PM-BPL)(13).

A live virus chick embryo vaccine—Flury-HEP virus(4) (Lederle) available commercially for immunization of cattle (HEP-EE).

A duck embryo inactivated vaccine—Duck embryo vaccine(14) (DE) (Eli Lilly) available commercially for human immunization.

According to the schedule shown in Table I, the HDCS vaccines were administered in 1 ml volume, and the HEP-EE vaccine in 0.5 ml volume by intramuscular inoculation into the thigh muscle. The DE vaccine was given subcutaneously in 0.5 ml quantity.

Serum neutralization test. Neutralization tests were performed by a method described by Koprowski and Johnson(15). A serum dilution was believed to contain antibodies if 2 or more mice in a group of 5 survived inoculation. The diluent was 2% normal inactivated calf serum in phosphate buffered saline (PBS). The same CVS pool of virus, stored in an aliquot at —70°C, was used for all tests at a dilution of 1 to 12,000 and contained about 50 LD_{50} for mice after addition of serum and incubation at 37°C for 90 minutes. The animals were observed daily for 2 weeks and the number of dead and sick recorded. Animals found dead during the first 4 days after inoculation were not considered as having died of rabies.

Results. Mouse potency test. The 4 vaccine preparations were submitted to the NIH Standard Potency Test(15) and the results shown in Table II indicate that they all passed the minimum requirements of the test set up as an 0.6 protection index.

Immunization of monkeys. With the exception of 2 animals in 2 experimental groups which died of type IV pneumococcal meningitis on the 15th and 17th day following inoculation, none of the remaining monkeys showed signs of either local or systemic reaction during the entire observation period. Splenectomy (see above) had no apparent effect on the outcome of the immunization.

No anti-rabies antibodies were detected in a 1:10 dilution of serum obtained from all animals before vaccination, but as shown in Fig. 1 and Table III, neutralizing antibodies were detected in all serum specimens drawn on the 7th day after vaccination. The highest level was observed in sera obtained from monkeys injected with HEP/HDCS vaccines and the lowest in sera drawn from the group

TABLE II. Results of NIH Potency Tests.

Type of vaccine	NIH test protection index	Titer in baby mice LD_{50}/.02 ml
HEP/HDCS	25.0	5.6
PN/BPL	3.3	—
HEP/EE	5.0	5.3
DE	1.0	—

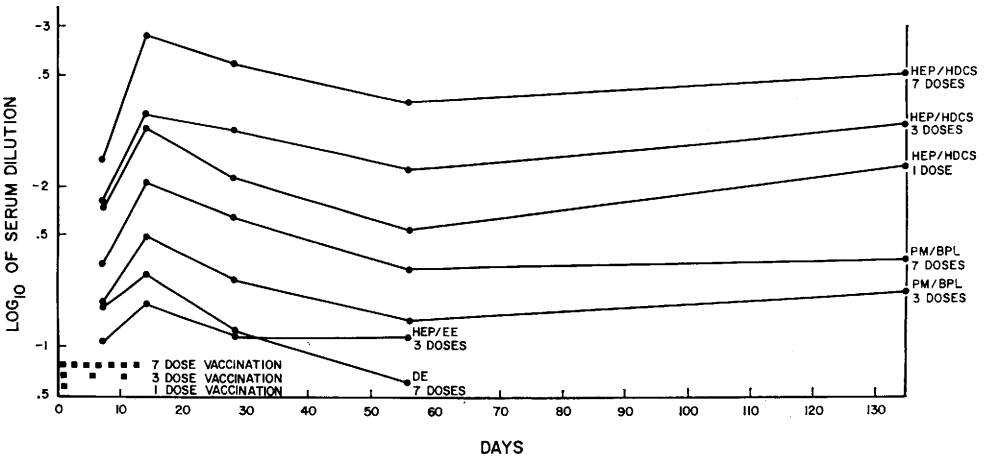

FIG. 1. Average neutralization titers of serum from monkeys vaccinated with different antirabies vaccines.

which received either HEP/EE or DE vaccines.

There was a considerable rise in concentration of antibodies in sera obtained from the HEP/HDCS groups on the 14th day after vaccination, and a more moderate rise in sera collected from the PM/BPL group. Only a slight change was noted in the blood drawn from the HEP/EE and DE groups which already had a low antibody concentration and, since tests on the 28th and 56th days showed only a barely detectable antibody titer (1/6 and 1/12, Table II), sera collected from these groups were not tested further.

There was a slight drop in the level of neutralizing antibodies between the 14th and 56th post-vaccination days in sera from monkeys that received the HEP/HDCS or PM/BPL vaccine, but surprisingly enough, sera obtained 135 days after vaccination from all the groups showed an apparent rise in antibody content rather than a reduction. Because of the inaccuracy inherent in the mouse neutralization test, this rise may be more apparent than real; nevertheless, the antibody concentration in the blood of the HEP/HDCS and PM/BPL groups did not decrease during the period between the 56th and 135th days after immunization, regardless of the dose schedule.

TABLE III. Antibody Response in Monkeys Immunized with Rabies Vaccine.

Vaccination		Neutralizing titer of rabies—antibodies in sera of monkeys					
		Days after vaccination					
Type of vaccine	No. of doses	1	7	14	28	56	135
HEP/HDCS	7	<10*	144	864	592	325	500
	3	<10	82	288	232	132	224
	1	<10	73	224	115	53	120
PM/BPL	7	<10	33	104	65	31	35
	3	<10	19	48	26	15	22
HEP/EE	3	<10	11	19	12	12	—
DE	7	<10	18	29	12	6	—

* Figures indicate average titers for each group of animals.

Discussion. The results shown in the preceding section indicate that the HEP-Flury virus of the 49th passage level in HDCS is a much better antigen than the same strain cultivated in fertile hens' eggs before adaptation to HDCS. The difference in the antigenicity of the two preparations cannot be attributed to the concentration of the infective virus since each monkey received an inoculum of either $10^{7.3}$ mouse LD_{50} of HEP/HDCS or $10^{7.0}$ mouse LD_{50} of HEP/EE (Table II) by the same route of inoculation.

It is, therefore, possible that the adaptation of HEP-Flury to HDCS resulted in a selection of virus particles of greater antigenicity as reflected both in the mouse potency test(13) and antibody response in monkeys. Also, it is conceivable that passage of the virus in a fibroblast culture of human origin may lead to a selection of virus particles which, in contrast to HEP/EE, are able to multiply in muscle tissue of primates

and possibly man, completely losing their neurovirulence at the same time(13).

On the basis of the results presented so far, it is impossible to discern which of the two mechanisms is responsible for the better antigenicity of HEP/HDCS. However, it should be emphasized that one injection of HEP/HDCS vaccine resulted in an immunogenic response greatly superior to the response observed after inoculation of 3 doses of HEP/EE vaccine.

Adaptation of the PM strain to HDCS seemed also to increase the antigenic properties of this strain. When propagated in HDCS and inactivated by BPL, the virus evoked a better antigenic response than when it was grown in duck embryo before inactivation by BPL. The immunogenic response of monkey to PM/BPL vaccine was even greater than their response after vaccination with living HEP/EE virus, but the antigenicity of PM/BPL vaccine was inferior to that of living HEP/HDCS virus.

Other investigators have noted that rabies virus showed increased antigenicity after cultivation in tissue culture. For example, Dean *et al*(10) observed that the HEP Flury strain grown in chick embryo *tissue culture* was antigenically superior to the same strain cultivated in fertile hens' eggs when the 2 were compared in the dog protection test. However, while the chick embryo vaccine passed the minimum requirements of the mouse potency tests, the tissue culture vaccine did not. Since Dean *et al*(10) did not use their virus for immunization of monkeys, it is difficult to compare their results with those reported here; but it should be noted that the greater antigenicity of the HEP/HDCS virus could be demonstrated either in the mouse potency tests or by evoking an antibody response in vaccinated monkeys.

Following adaptation of fixed strains of rabies virus to primary hamster kidney culture, Kissling and Reese(16) and Fenje(17) were able to demonstrate antigenicity of the formalin inactivated vaccine in mice and rabbits. However, the results of protection tests in guinea pigs with the same type of vaccine were not as satisfactory as with the commercially available Flury chick embryo vaccine (Ott and Heyke)(18). Abelseth(19, 20) succeeded in propagating the hamster kidney-adapted virus in primary pig kidney culture but the resulting strain retained its neurovirulence for laboratory rodents, dogs, and cats and was found to be antigenic in guinea pigs, dogs, and cattle after administration as live virus vaccine.

Although the neurovirulence of this strain precluded its use in man as live virus vaccine, none of the other strains propagated in primary cultures of animal tissue could be considered suitable for immunization of man because of the possible presence of contaminating viruses in the vaccine preparations (21,22).

"Native" viruses which could contaminate the vaccine preparation have been recovered from primary tissue culture of many animal species(23). Since these viruses may not necessarily be inactivated by the concentration of the virucidal agent employed, it is imperative to reconsider the problem of production of either live or inactivated vaccine in primary explants of animal tissue. In contrast, the human diploid cell strain has been found to be free from extraneous viruses which could cause either cytopathic effect or interference phenomenon in homologous and heterologous tissue culture systems(24). Thus, vaccines prepared in HDCS may have some advantage over vaccines prepared in other cell systems.

Taking all these facts into consideration, an extensive study of the immunogenic capacity of HEP/HDCS in man should be contemplated.

Summary. The antigenicity of 2 strains of rabies virus (Flury HEP and Pitman-Moore) propagated in a Human Diploid Cell Strain and used as live and inactivated vaccine was studied in Rhesus monkeys. It was found that these strains were much more antigenic than the same strains of virus propagated in embryonated avian egg before adaptation to tissue culture. The use of this tissue culture vaccine in man should be contemplated.

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Significance of Antibody to *Mycoplasma hominis* Type 1 as Measured by Indirect Hemagglutination. (30049)

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Mycoplasma pneumoniae has been established as the etiologic agent of naturally occurring cold agglutinin-positive primary atypical pneumonia, and this mycoplasma may also cause febrile upper respiratory illness(1). In addition, experimentally infected volunteers have been observed to develop bullous myringitis(2). Although *M. pneumoniae* is the only mycoplasma species that has been conclusively shown to be a human pathogen, recent evidence suggests that another species may be capable of causing human disease. In this laboratory, *M. hominis* type 1 has been isolated more frequently from persons with respiratory illness than from healthy persons, and we have demonstrated that *M. hominis* type 1 is capable of producing exudative pharyngitis in experimentally infected volunteers(3). The detection of antibody and measurement of antibody rises in these volunteers was accomplished only by means of the indirect hemagglutination (IHA) technique, due to its greater sensitivity in comparison with other serological methods. Information concerning the sensitivity and specificity of the IHA technique and the speed of development, persistence and protective effect of IHA anti-

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