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BIOLOGICAL MODIFICATION OF RABIES VIRUS AS A RESULT OF ITS ADAPTATION TO CHICKS AND DEVELOPING CHICK EMBRYOS

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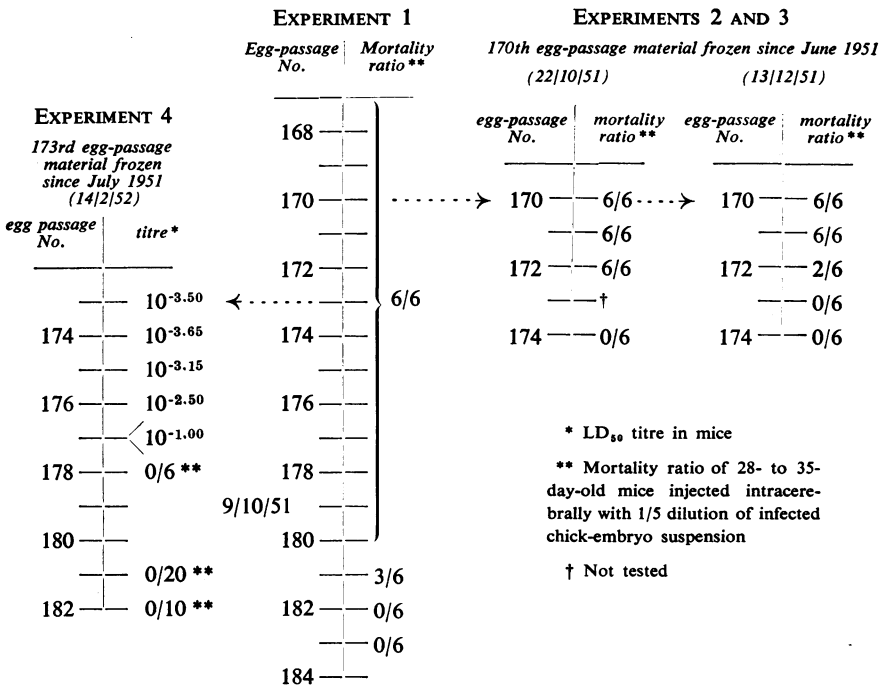
SYNOPSIS

This article describes experiments indicating a change in pathogenicity for laboratory animals of the Flury strain of rabies virus at high egg passages. Factors such as dilution of virus, number of egg passages, age of animals, and route of inoculation are taken into account. The results of the author's investigations indicate that living chick-embryo-adapted virus can be used both as a vaccine administered before exposure to rabies virus, and as an adjunct to antiserum in the protective treatment of animals after exposure.

On 29 March 1939, a girl named Flury died of rabies in Georgia, USA, after an illness of four days' duration. She had been exposed to the licks of a rabid dog some days before signs of sickness were noted. The dog died five days before the girl became ill. She did not receive Pasteur treatment. The autopsy was performed by Dr. Harald Johnson of the Rockefeller Foundation, who found street virus present in the central-nervous-system tissues and the lachrymal and salivary glands by injecting tissues obtained at the autopsy into white mice.[4] Dr. Johnson also injected brain-tissue suspension into one-day-old chicks. It took 30 days for the first passage of this material to cause signs of paralysis in the inoculated birds. Serial passages in this host resulted in a shortening of the incubation to six days, and Dr. Johnson was able to pass this strain for 136 chick-brain passages.[3]

I received this material in 1945 and, after two consecutive passages in one-day-old chicks, adapted it to the developing chick embryo.[3] At the low egg-passage levels the virus was found to become innocuous for most mammals injected parenterally.[1, 3] After prolonged serial passage of the Flury strain in the developing chick embryo, another profound change in its pathogenicity has become apparent, as is shown in the following tabulation :



It will be noticed from the results of Experiment 1 that a change in the pathogenic properties of the virus became apparent at the 180th egg-passage level. A 20% suspension of infected chick embryo representing the 182nd egg-passage failed to elicit signs of illness in intracerebrally inoculated mice 28-35 days old. At first it was suspected that the virus was lost in the course of transfer from one egg generation to another, but, after repeated trials and repeated " loss " of the virus, guinea-pigs were inoculated intramuscularly with the " apathogenic " suspension, and subsequently challenged with street virus. The results seemed to show that the material, which had no pathogenic properties for intracerebrally inoculated mice, still remained an excellent antigen for the immunization of guinea-pigs.

This apparent change in pathogenic properties was, therefore, further investigated (Experiments 2 and 3). Chick-embryo suspensions representing the 170th egg-passage were thawed on two separate occasions, and two series of egg passages were started. The results were identical : between the 172nd and the 174th chick-embryo generation the material had become apathogenic for young adult mice injected intracerebrally with a 20% suspension originating from either of the egg-passage series. More quantitative studies were performed in Experiment 4, with results indicating that the loss of pathogenicity for intracerebrally inoculated mice occurred quite rapidly,

and was preceded by a decrease in the median lethal dose (LD_{50}) titre for one or two generations. In the same experiment, the " presence " of virus at the 181st and 182nd egg-passage levels was confirmed by the resistance of guinea-pigs injected with the material and subsequently challenged with street virus.

Since resistance of mice to viral infection has been shown to increase with age, attempts were made to determine whether mice younger than 28 days of age would still remain nonsusceptible to the pathogenic properties of the high-egg-passage virus, or what we will call the HEP Flury strain. The results indicate that mice three and eight days old were found to be highly susceptible to intracerebral infection. A break in susceptibility to infection was found to occur between the ages of eight and 14 days; mice of the latter age were found to be completely resistant to intracerebral inoculation with HEP Flury virus. As indicated in table I, 14-day-old mice remaining symptom-free after original inoculation were challenged intracerebrally with street virus four weeks later. The results indicated that the first injection of HEP Flury virus induced resistance to challenge inoculation without causing clinical signs of sickness in mice.

TABLE I. INFLUENCE OF AGE OF MICE UPON SUSCEPTIBILITY TO INTRACEREBRAL INOCULATION OF HIGH-EGG-PASSAGE FLURY STRAIN

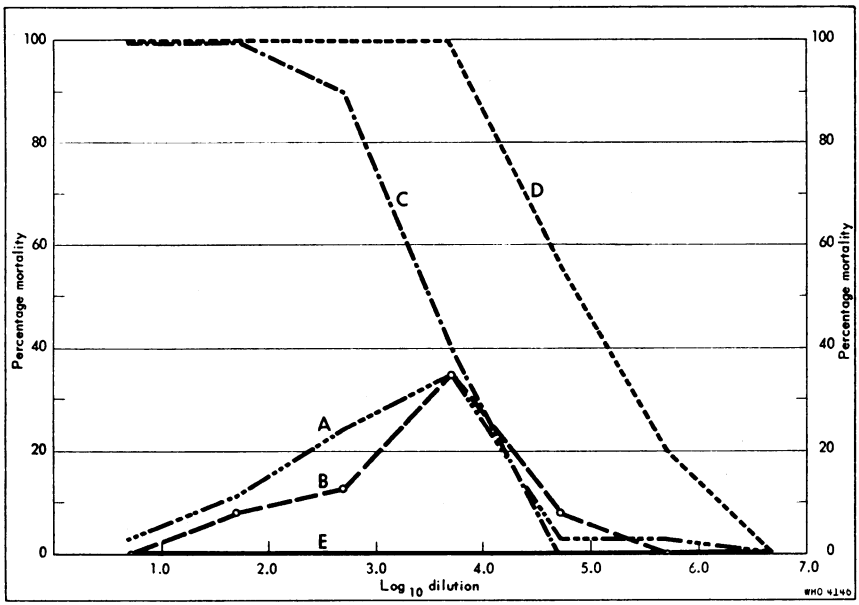
Egg passage	Age of mice (days)		Mortality ratio of animals injected with dilutions * of Flury strain (A) and later challenged ** with street virus (B)								
			0.70	1.40	2.10	2.80	3.50	4.20	4.90	5.60	6.30
194	3	A	.	.	.	.	8/8	7/7	0/0	2/11	0/12
		B	.	.	.	.	—	—	—	9/9	11/12
	8	A	.	8/8	8/8	8/8	8/8	5/8	1/7	0/4	.
		B	.	—	—	—	—	0/3	6/6	4/4	.
	14	A	0/7	0/8	0/8	.	.	.	.	.	.
		B	0/7	0/8	0/8	.	.	.	.	.	.

* Expressed as logarithms

** By intracerebral route with 6.75 LD_{50} of virus

Titrations of infected embryo-suspensions representing the 178th to 205th egg-passage of the Flury strain were made in six species of animals (including baby and adult mice) and the results are shown in fig. 1. In replicate experiments (not less than two for each species), animals were injected intracerebrally with serial tenfold dilutions of the virus. The mortality-rates indicate that adult mice, rabbits, and dogs seem to be equally

FIG. 1. PERCENTAGE MORTALITY OF TEST ANIMALS INOCULATED INTRACEREBRALLY WITH DILUTIONS OF HEP FLURY VIRUS



A Hamsters C Monkeys
B Guinea-pigs D Baby mice
E Dogs, rabbits, adult mice

nonsusceptible to intracerebral inoculation of the HEP Flury virus. Rhesus monkeys still seemed to remain highly susceptible to intracerebral inoculation with the HEP Flury virus, the mortality end-point of the virus being only slightly lower than that obtained in baby mice, the most susceptible species.

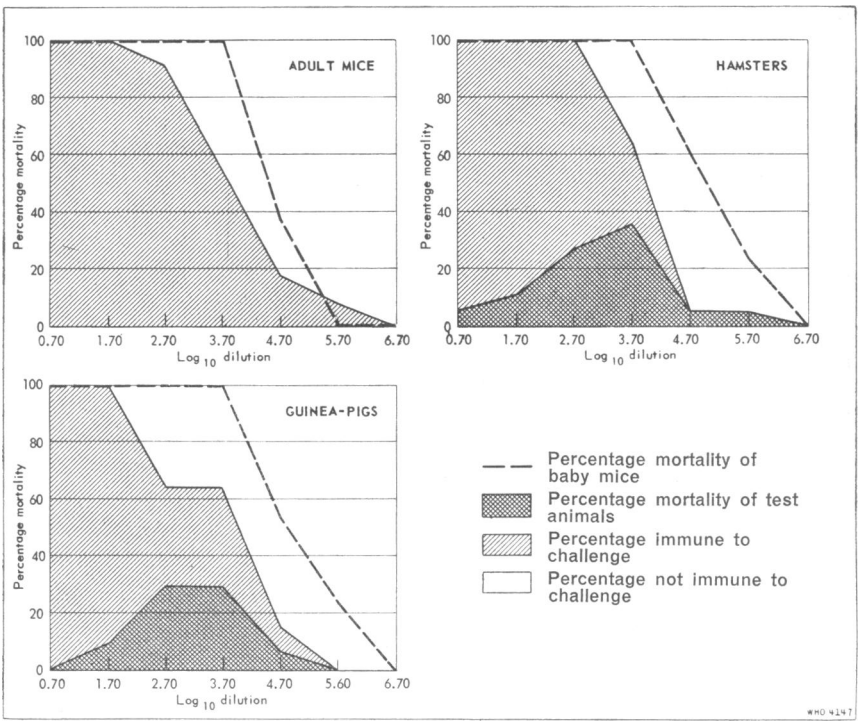
The results obtained in hamsters and guinea-pigs are, perhaps, of greatest interest since they indicate that, had only a concentrated suspension of infected chick-embryos been used in this study, both species might have been considered to represent nonsusceptible hosts. That this was not the case was clearly demonstrated by the fact that some animals died when infected with dilutions higher than that of log 0.7 of the infectious material. The mortality peak was observed at the dilution level of log 3.7; from then on, the mortality curve sloped rather rapidly downward, reaching negligible proportions at a dilution of log 4.7, and zero at that of log 6.7.

Judging from the mortality curves for hamsters and guinea-pigs, it may be assumed that an infectious process took place in those animals injected with the lower dilutions of the virus, although clinical signs of illness were lacking. In order to verify this assumption, all animals remaining symptom-free after the original inoculation were challenged intracerebrally 60 days

later with street virus. Also included as controls in the challenge experiments were the adult mice.

The results (see fig. 2) indicate that injection of HEP Flury strain into the central-nervous-system tissue of hamsters induced a state of resistance to subsequent challenge with street virus. The rate of resistance drops at that dilution of Flury strain which causes death in most animals. Though

FIG. 2. RESISTANCE OF HAMSTERS, GUINEA-PIGS, AND ADULT MICE TO CHALLENGE WITH STREET VIRUS



it runs slightly behind, the resistance curve seems to parallel the death curve caused by the Flury inoculum in baby mice. It may be seen that similar results were obtained in the experiments with guinea-pigs. Adult mice which originally remained symptom-free after inoculation with Flury strain showed a high degree of resistance to challenge with street virus. However, the pattern of the resistance curve again seemed to parallel the death curve caused by the Flury inoculum in baby mice.

The results described thus far seem to indicate that the HEP Flury virus represents a mixed viral population consisting of particles still pathogenic for intracerebrally-injected hamsters and guinea-pigs, and of particles deprived of their pathogenic properties. The presence of the former becomes

TABLE II. ATTEMPTS TO PRODUCE INTERFERENCE BETWEEN THE 202nd EGG-PASSAGE OF THE FLURY STRAIN AND THE STREET, RODENT-FIXED, AND LOW-EGG-PASSAGE FLURY VIRUSES

Strains of virus	Dilutions made in	Mortality ratio of animals injected intracerebrally with dilutions* of virus							LD_{50} titre of virus
		1.00	2.00	3.00	4.00	5.00	6.00	7.00	
Street	HEP Flury	.	6/6**	6/6	6/6	5/5	0/5	0/4	5.50
	Normal chick embryo	.	5/5	6/6	5/5	2/6	1/6	0/5	4.90
Rodent-fixed	HEP Flury	.	5/5**	5/5	5/5	5/5	5/5	3/6	7.00 or >
	Normal chick embryo	.	5/5	5/5	5/5	5/5	5/5	2/6	6.75 or >
Flury, 68th egg-passage	HEP Flury	0/4†	0/4	0/3	0/4	0/4	.	.	< 1.00
	Normal chick embryo	4/4	4/4	2/4	2/4	3/4	.	.	4.35 or >

* Expressed as logarithms
** Young adult mice aged 21-28 days
† Hamsters aged 60-90 days

manifest only after the HEP Flury virus is used at higher dilution levels. This phenomenon, repeatedly observed in titrations in hamsters and guinea-pigs, may be caused by the interference of particles nonpathogenic for hamsters and guinea-pigs with those that still remain pathogenic. In order to elucidate this phenomenon, experiments were planned in hamsters injected with serial tenfold dilutions of Flury virus at the 68th egg-passage level, which is still highly pathogenic for this species. In this experiment two diluents were used : (*a*) a 20% chick-embryo suspension infected with the 202nd egg-passage of Flury virus, and (*b*) a normal uninfected chick-embryo suspension. The same diluents were used in two other experiments in which street and rodent-fixed strains of rabies were used for titration purposes. These mixtures were injected into animals without incubation. The results indicate that the HEP Flury virus possesses a remarkably strong ability to interfere with the infectious process induced in hamsters by intracerebral inoculation of Flury strain at the 68th egg-passage level. This interfering ability was not manifest when either street or rodent-fixed virus was used as inoculum.

These results may indicate that the assumption that a mixed viral population exists in the HEP Flury virus is not without basis. It is possible to conceive that a small number of virus particles which are still pathogenic for hamsters and guinea-pigs remain in the virus preparation, and that their pathogenic properties become manifest only when that segment of the virus population which is nonpathogenic for hamsters and guinea-pigs is diluted beyond the interfering point.

It then seemed to be of interest to investigate whether the virus isolated from the brain tissue of the hamsters which died after intracerebral inoculation of HEP Flury virus would remain pathogenic only for baby mice or whether it would regain its virulent properties for adult mice. In table III are summarized the results of experiments in which baby and adult mice were injected simultaneously with suspensions of brain tissue from hamsters which died after inoculation with the HEP Flury virus. It may be seen that ten hamster brains yielded virus infectious for baby-mouse brains. In not a single instance was this virus pathogenic for young adult mice.

TABLE III. COMPARATIVE PATHOGENIC PROPERTIES FOR BABY AND YOUNG ADULT MICE OF THE VIRUS ISOLATED FROM BRAIN TISSUE OF HAMSTERS INOCULATED INTRACEREBRALLY WITH HEP FLURY STRAIN

Species	Experiment No.	Animal No.	Mortality ratio of baby and adult mice injected with brain suspension of animals originally inoculated with dilutions * of virus							
			0.70		1.70		2.70		3.70	
			A	B	A	B	A	B	A	B
Hamster	199	1	.	.	3/6	0/6	6/6	0/5	.	.
		2	.	.	.	.	1/5	0/6	.	.
		3	.	.	.	.	7/7	0/6	.	.
	204	1	6/6	0/6	6/6	0/6	6/7	0/6	7/7	0/6
		2	.	.	.	.	6/6	0/6	4/4	0/6

A = 3- to 5-day-old mice
B = 21- to 28-day-old mice

* Expressed as logarithms

In attempts made to produce virus forms pathogenic for adult mice by serial passage through adult-mouse brains, it was determined that a virus still pathogenic for baby mice is present in the adult-mouse brain only until the fifth day after inoculation. We then proceeded to outline an elaborate trial in which serial tenfold dilutions of the Flury strain representing the 194th egg-passage of the virus were injected intracerebrally into groups of from seven to eight adult mice per dilution, and at the same time the inoculum was titrated in baby mice. Two or three adult mice injected with the respective viral dilutions were sacrificed five days later, and pools of their brain tissue were made, this time into a 10% suspension, and subinoculated into groups of adult and baby mice, respectively. It may be seen that none of the animals showed signs of illness in the course of the ten passages, although the virus was present throughout the experiments.

TABLE IV. ATTEMPTS TO INCREASE THE PATHOGENIC PROPERTIES OF THE HEP FLURY STRAIN THROUGH SERIAL BLIND PASSAGES IN ADULT MOUSE BRAIN

Dilution of the 194th-egg-passage virus	Titration results of the original inoculum	Mortality ratio of baby and young adult mice injected with brain tissue of adult mice at each passage level. Mortality ratio after challenge of survivors with street virus						
		passage no.						
		1		5		10		
	A	B	A	B	A	B	A
10^{-3}	0/7	3/3	0/8	7/7	0/7	5/5	0/6
	0/5 *	—	0/6	—	0/5	—	0/5

A = 21- to 28-day-old mice
B = 3- to 5-day-old mice

* This line gives challenge results.

In several preliminary titrations of the HEP Flury virus, it was observed that sometimes material obtained after one passage through baby-mouse brain caused sickness in young adult mice when injected intracerebrally. It seemed to be interesting to know if this reversion of properties of HEP Flury virus remained permanent after further passage through eggs, or if its pathogenicity for young adult mice was again lost. Each of the next three experiments was conducted along the same pattern. Material representing the HEP Flury virus was injected into baby mice in a dilution of log 0.7. When these mice showed signs of sickness, they were sacrificed and a 10% suspension of their brain tissue was injected into fertile hens' eggs by the yolk-sac route, into young adult mice by the intracerebral route, and, in two experiments, baby mice by the intracerebral route. If and when the adult mice showed signs of sickness, they were sacrificed and their brain tissue was injected into developing chick embryos.

In Experiment 1 (see fig. 3), direct inoculation from baby-mouse brain into the yolk-sac of the developing chick embryo yielded a virus which was pathogenic for young adult mice after the first egg-passage but which lost that characteristic after two subsequent egg-passages. In contrast, the series which was started after an intervening passage through adult-mouse brain remained pathogenic for adult mice throughout 18 subsequent egg-passages, when the experiment was terminated.

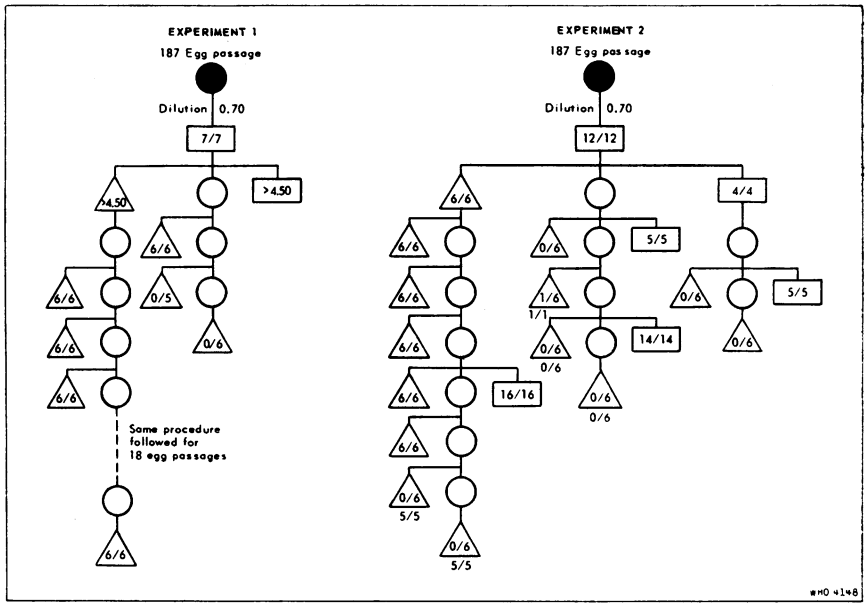
In Experiment 2 (see fig. 3), two egg-passage series started from one and two baby-mouse brain passages gave the same results—namely, that only

virus pathogenic for baby mice was propagated. The same material injected into adult mice failed to elicit signs of sickness, but the symptom-free animals remained resistant to challenge inoculation with street virus. The remaining egg-passage series initiated from the adult-mouse brain passage yielded a virus which remained pathogenic for adult mice for five consecutive egg-passages, after which the virus seemed to be lost since it failed to elicit either signs of sickness or immunological response on subsequent challenge inoculation.

Results of the previous experiments indicate that intracerebral inoculation of the HEP Flury strain into mice 14 days of age or over induced resistance against challenge with street virus. It seemed to be of interest to investigate whether this resistance was conditioned by the persistence of the " masked " virus in the susceptible cells of the central nervous system, or by the presence of antibodies formed in mice through an antigenic stimulus.

Groups of mice were injected intracerebrally with serial tenfold dilutions of chick-embryo material representing the 194th egg-passage of the Flury

FIG. 3. CHANGE IN THE PATHOGENIC CHARACTERISTICS OF HEP FLURY STRAIN AFTER PASSAGE THROUGH BABY- AND ADULT-MOUSE BRAIN



Egg

6/6 Mortality ratio of baby mice

3/6 Mortality ratio of adult mice after original inoculation (inside triangle) and after re-challenge (outside triangle)

0/3

strain. Fourteen days later, six mice from each group were exsanguinated through heart puncture, and the undiluted serum from each mouse was submitted to a neutralization test against fixed virus. At the same time, parallel groups were challenged intracerebrally with 2,000 LD_{50} of street virus. The same procedure was followed on the 21st day after inoculation of HEP Flury strain.

The results of challenge, and those of the neutralization test, are summarized in table V. It may be observed that, within the framework of experimental error, the presence of homologous antibodies correlated exactly with resistance to challenge inoculation. This correlation existed whether the time-interval between immunization with HEP Flury strain and challenge inoculation was 14 or 21 days. These data seem to rule out the possibility that interference plays any role in effecting a resistant state in mice to infection induced by inoculation with HEP Flury strain.

TABLE V. CORRELATION BETWEEN RESISTANCE AND SEROLOGICAL EVIDENCE OF IMMUNITY INDUCED IN MICE BY THE 194th EGG-PASSAGE OF FLURY STRAIN

Dilution * of Flury strain inoculated intracerebrally	Ratio of mice : (A) showing serum-neutralizing antibodies, and (B) surviving challenge ** with street virus, at days after inoculation with Flury strain			
	14		21	
	A	B	A	B
0.70	6/6	6/6	6/6	6/6
1.70	6/6	6/6	6/6	6/6
2.70	6/6	4/5	6/6	6/6
3.70	2/6	3/6	5/6	3/5
4.70	1/6	1/6	0/6	1/6
5.70	1/6	1/6	3/6	0/6

* Expressed as logarithms

** By intracerebral route with 2,000 LD_{50} of virus

Table VI summarizes the results of experiments in which groups of guinea-pigs were inoculated with Flury strain representing either the 51st or the 178th egg-passage level. Starting with a 20% suspension of infected chick embryo, serial fourfold dilutions stemming from each passage level were injected simultaneously into groups of Swiss albino mice by the intracerebral route. It will be noted that the 51st-egg-passage virus produced a titre of 4.5, whereas the 178th-egg-passage inoculum failed to elicit any signs of sickness in the inoculated animals. Three weeks after immunization, all vaccinated guinea-pigs and ten untreated controls were challenged intramuscularly with street virus in the form of a 10% suspension of canine

TABLE VI. RESULTS OF COMPARATIVE IMMUNIZATION OF GUINEA-PIGS WITH LOW- AND HIGH-EGG-PASSAGES OF THE FLURY STRAIN

Flury strain		Mortality ratio of challenged * guinea-pigs immunized with dilutions ** of vaccine					
egg passage	mouse LD_{50} titre †	none (controls)	1.30	1.90	2.50	3.10	3.70
none (controls)		10/10					
51	4.5		1/4	0/4	1/5	2/5	2/5
178	<0.70		0/5	0/5	0/5	4/5	5/5

* Street virus (canine salivary gland)
** Dilutions expressed as logarithms
† Based on intracerebral inoculation ; expressed as negative logarithms

salivary gland, a preparation found to be lethal for all control animals. Although it may appear from the results of the test that the Flury strain may be slightly more antigenic at the 51st egg-passage level, the difference in the numbers of dead animals is too small to be considered significant. In several subsequent experiments, no difference whatever was observed between the antigenic power of the low-egg-passage virus and that of the high-passage virus.

TABLE VII. IMMUNIZATION OF DOGS WITH HEP FLURY STRAIN OF RABIES VIRUS

Egg passage of Flury strain	Mortality ratio of dogs challenged * with street virus
180	3/10
187	0/25
none (controls)	15/19

* Challenged 30 days after vaccination by intramasseter inoculation of NYC strain of street virus

At present [1953] the canine antirabies vaccine is prepared from material derived from the 40th to 50th egg-passage level of the Flury strain. In view of the marked change in pathogenic properties of the Flury strain at the high egg-passage level, an attempt was made to determine the antigenic power of the HEP virus in dogs. Groups of dogs were injected intramuscularly with 3.3 ml amounts of a 33% suspension of chick embryo, representing the 180th to 187th egg-passage of the Flury strain rehydrated from the dried state. One month later, all vaccinated animals and 15 untreated controls were challenged by intramasseter inoculation with 1/40 dilutions of canine salivary

gland infected with the street strain of rabies virus. The results of the test indicate that the HEP virus is as excellent an immunizing agent as the low-egg-passage virus. The difference in the mortality ratios between the vaccinated dogs and the untreated controls was large enough to be considered of statistical significance.

Since experiments in cattle have shown the low-egg-passage virus to be a safe immunizing agent, tests in the same species were undertaken with the HEP Flury virus. The 185th egg-passage of the Flury strain was injected into groups of calves aged from six to eight months. The vaccinated animals were bled 33 days later and immediately thereafter were challenged with street virus injected into the masseter muscles. The respective sera were submitted to neutralization tests against 50-100 LD_{50} of fixed virus. The ratios of animals showing the presence of neutralizing antibodies as compared to the ratios of animals surviving challenge inoculation with Flury virus are shown in table VIII. It may be concluded that three different dilutions of street virus used for challenge purposes resulted in no differences in the mortality ratios of either control or vaccinated animals. It may be further observed that, in most instances, there was a close correlation between the ratios of those animals which developed neutralizing antibodies and the ratios of those which were resistant to challenge inoculation. Furthermore, it is quite clear that the results obtained with the inoculum of the 3-ml dose of the Flury strain were as good as those obtained with a dosage of 15 ml.

TABLE VIII. CORRELATION BETWEEN RESISTANCE AND SEROLOGICAL EVIDENCE OF IMMUNITY INDUCED IN CATTLE BY THE 185th EGG-PASSAGE OF FLURY STRAIN

Log of dilution of challenge virus	Ratio of calves : (A) showing serum—neutralizing antibodies, and (B) surviving challenge with street virus, after immunization with different amounts (ml) of Flury strain					
	3		15		none	
	A	B	A	B	A	B
1.50	3/3	3/3	3/3	3/3	0/2	0/2
1.95	3/3	2/3	3/4	3/4	0/3	0/3
2.40	2/2	2/2	2/3	2/3	1/3	0/3
Total	8/8	7/8	8/10	8/10	1/8	0/8

In order to obtain some information on the immunogenic properties of the HEP Flury virus in primates, four chimpanzees less than two years of

age were immunized with chick-embryo material representing the 194th egg-passage of the virus. All animals received the equivalent of two dog doses (6.6 ml of a 33% suspension) injected deep into the muscles of the thigh. Two of the chimpanzees received the same amount of virus in the alternate limb ten days later. All chimpanzees were bled at the time intervals shown in table IX and the individual sera were submitted to neutralization tests aginst 470 LD_{50} of virus. The results of the test indicate that no apparent difference in the immunogenic response could be noted between those animals which received one, and those which received two, injections of the Flury strain. In three out of four chimpanzees, neutralizing antibodies were observed on the 21st day after immunization, and all the animals had neutralizing antibodies 28 and 35 days after inoculation with Flury strain. While the level of the neutralizing antibodies was not very high, in view of accumulated evidence it may be considered to be sufficient for protective purposes.[2]

TABLE IX. IMMUNIZATION OF CHIMPANZEES WITH 194th EGG-PASSAGE OF FLURY STRAIN: RESULTS OF NEUTRALIZATION TEST *

Number of injections of Flury strain	Minimum protective serum titres** obtained days after immunization		
	21	28	35
1	0.75	1.00	1.00
	0.65	0.65	> 1.00
2†	0.40	0.65	0.40
	0	0.40	> 1.00

* Against 470 LD_{50} of virus
** Expressed as negative logarithms
† The second injection was given 10 days after the first.

While our emphasis thus far has been towards a means of control of rabies through the immunization of animals, our ultimate objective is, of course, the application of these findings to the treatment of human beings who have been exposed to the infection. Progress in this direction required further investigations in a laboratory animal with an incubation period and susceptibility thought to be comparable to man's. Dogs, as natural hosts for rabies infection, were ideally suited for this purpose and, since previous studies have shown that antiserum is of great value in preventing rabies after exposure, the use of vaccine combined with antiserum was considered in the following experiments. Adult dogs and puppies born and reared in our laboratories and known to be non-immune to rabies were carefully

weighed, and injected with different dilutions of street virus. Adult dogs received 0.06 ml bilaterally into the massater muscle, whereas the more highly susceptible puppies were injected with 0.3 ml in the muscles of the hind leg. Twenty-four hours later the animals were divided into several experimental groups and treated as shown in table X. Antiserum was administered in an undiluted form of the same concentration employed in human prophylaxis : 0.25 ml subcutaneously per pound of body-weight. The Flury strain was injected in the form of a vaccine, each injection representing one immunizing dog-dose inoculated into the posterior muscular tissue of the hind leg.[a]

TABLE X. PROTECTIVE TREATMENT OF ADULT DOGS WITH RABIES ANTISERUM AND/OR FLURY STRAIN VACCINE AFTER EXPOSURE TO STREET VIRUS

Dilution of street virus	Treatment		Mortality ratio	Day of death
	antiserum 24 hours after exposure	vaccine days after exposure		
1/100	yes	none	6/10	15-83
	yes	2, 6, 9	1/10	16
	none	1, 4, 7	7/10	12-24
Controls	none	none	8/10	12-26

The animals were observed for one year. Every dog which died was autopsied and its brain and salivary-gland tissues were subinoculated into mice. Only when virus was isolated was the animal considered to be dead of rabies.

The results are shown in tables X, XI, and XII : the mortality ratio of adult dogs exposed to 1/100 dilution of street virus was similar to that of those animals which received Flury strain alone after exposure. No differences in incubation period were observed in these two groups. The effect of antiserum alone was noticeable only in a prolonged incubation period. Strikingly different results were obtained in dogs which received combined treatment of antiserum and Flury strain, since only one animal out of ten succumbed to rabies, dying 16 days after exposure.

Curiously enough, a change in street virus dilution to 1/1,000 as exposure source failed to influence the mortality ratio of untreated control dogs.

[a] 3.3 ml of a 33% suspension of chick-embryo tissue infected with the 50th egg-passage of the virus

TABLE XI. PROTECTIVE TREATMENT OF ADULT DOGS WITH RABIES ANTISERUM AND/OR FLURY STRAIN VACCINE AFTER EXPOSURE TO STREET VIRUS

Dilution of street virus	Treatment		Morta ity ratio	Day of death
	antiserum 24 hours after exposure	vaccine days after exposure		
1/1,000	yes	none	2/10	39, 49
	yes	2, 6, 9	1/10	13
	none	1, 4, 7	5/10	17, 17, 18, 19, 24
Controls	none	none	8/10	14-27

Again, eight out of ten animals died of rabies after incubation periods only slightly longer than those in the group injected with 1/100 dilution. The effect of treatment with Flury strain alone is difficult to evaluate because of the small number of animals and the relatively slight difference in mortality ratios. The action of antiserum alone was more pronounced than in the preceding group. The two animals which eventually died of rabies did so after incubation periods of 39 and 49 days, the longest in this group. Combined treatment with antiserum and Flury gave the same excellent results as in the preceding group.

TABLE XII. PROTECTIVE TREATMENT OF PUPPIES WITH RABIES ANTISERUM AND/OR FLURY STRAIN VACCINE AFTER EXPOSURE TO STREET VIRUS

Dilution of street virus	Treatment		Mortality ratio	Day of death
	antiserum 24 hours after exposure	vaccine days after exposure		
1/40	yes	none	4/10	16, 19, 21, 28
	yes	2, 6, 9	2/10	14, 30
	none	1, 4, 7	8/10	14-21
Controls	none	none	8/10	16-94

The mortality ratios of untreated puppies were the same as those of adult dogs. No effect of treatment with Flury strain alone after exposure was observed. Antiserum alone displayed a beneficial effect, but again the

group of puppies which received antiserum and Flury strain fared best, since only two out of ten animals died of rabies.

Our results over the past five years thus indicate that living chick-embryo-adapted rabies virus can be used both as a vaccine administered prior to exposure to rabies virus, and as an adjunct to antiserum in the protective treatment of animals after exposure. Although specific application of the results of our studies in animals cannot be made to the field of human prophylaxis, we may be optimistic that the foundation has been laid for the conquest of this tragic disease.

RÉSUMÉ

L'auteur rappelle l'origine de la souche de virus Flury, isolée en 1939 du système nerveux central, des glandes salivaires et lacrymales d'une victime de la rage, n'ayant reçu aucun traitement. Une suspension de tissu cérébral fut inoculée en série à des poulets âgés d'un jour. La période d'incubation, de trente jours au premier passage, s'abaissa à six jours après de nombreux passages. En 1945, l'auteur a adapté ce virus à l'embryon de poulet. Au cours des passages en série, la pathogénicité du virus se modifia considérablement. Entre le 172e et le 174e passage sur œuf, le virus (suspension à 20 % de tissu embryonnaire) ne provoquait plus de symptômes morbides chez les souris de 28-35 jours inoculées par voie intracérébrale. Pourtant, il gardait ses propriétés immunogènes pour le cobaye. L'analyse de ces phénomènes montra que la perte de la pathogénicité était rapide, et qu'elle était précédée d'une baisse de la dose létale minimum. La résistance des souris au virus HEP (high egg passage = nombre élevé de passages sur œuf) se manifestait dès l'âge de 14 jours. Les souris plus jeunes restaient sensibles à l'agent infectant. Les lapins et les chiens, comme les souris adultes, étaient résistants. Les singes Rhésus, par contre, ainsi que les souriceaux étaient très sensibles. Les hamsters et les cobayes, résistants à de fortes concentrations de virus HEP, se montrèrent sensibles, en revanche, à des dilutions plus élevées. Ce fait, apparemment paradoxal, suggère que le virus Flury HEP représente une population de virus, constituée, d'une part, de particules pathogènes pour les hamsters et les cobayes inoculés par voie intracérébrale et, d'autre part, de particules avirulentes. Ces dernières, par une interférence avec les particules virulentes, une sorte d'inhibition, les empêcheraient de manifester leur pathogénicité. Celle-ci n'apparaîtrait que lorsque la dilution du matériel infectant a été poussée assez loin pour que l'interférence des particules avirulentes devienne impossible.

L'auteur étudie ensuite l'effet prophylactique et thérapeutique sur le chien du vaccin souche Flury, de ce vaccin associé au sérum antirabique et du sérum seul. Il conclut, d'après des observations portant sur cinq ans, que le virus rabique vivant, adapté à l'embryon de poulet peut être administré avec succès comme vaccin préventif avant la morsure, et comme complément du traitement sérothérapique après morsure. Bien que les résultats des expériences sur l'animal ne puissent être directement appliqués à la prophylaxie humaine, l'auteur estime qu'ils peuvent constituer les fondements de méthodes qui doivent permettre, à l'avenir, de vaincre la rage.

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