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STUDY OF ANTIRABIES IMMUNIZATION OF MAN

Observations with HEP Flury and Other Vaccines, with and without Hyperimmune Serum, in Primary and Recall Immunizations

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SYNOPSIS

Detailed results are presented of primary immunizations of 387 persons with various courses of HEP Flury vaccine and of 54 persons with Harris- or Semple-type vaccines. Antibody response to HEP Flury vaccine was at least as rapid as that to the conventional type, but fell short in uniformity and level of response. The most promising course involved a 4-dose schedule, intradermal alone or combined with intramuscular, at 5-day intervals. A similar subcutaneous course of Semple vaccine yielded results completely equivalent to those of a 14-dose course of Harris vaccine. It is concluded that, although living, the HEP Flury virus does not multiply in man and that its lesser antigenic potency, as compared with Semple or Harris vaccines, is due to its relatively small content of viral antigen.

Further evidence has been obtained that hyperimmune serum may exert a slight suppressive effect on active response, but the opinion is expressed that, with vaccines of full potency, this will not be of practical significance.

Restimulation of immunity by a booster dose of HEP Flury vaccine was studied in 64 experimentally immunized persons and in 136 persons with history of previous Pasteur treatment. In both instances small intradermal inocula were as effective as larger intramuscular inocula in recalling pre-existing immunity.

Study of recipients of Pasteur treatment indicated that antibody commonly persists for at least 5 years after a single course and for 15 or more years after re-treatment. It was also observed that the ability to respond to a booster of HEP Flury vaccine persists for at least 25 years. The response elicited by the booster is prompt and is usually at least equal to that resulting from a full primary course. The suggested conclusion is that previously treated persons need not receive more than a single booster on re-exposure, and that Pasteur treatment provides a solid basis for long-sustained immunity.

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Originally isolated from a fatal human case and carried in brain-to-brain passage in day-old chicks by Dr Harald N. Johnson, the Flury strain of rabies virus was adapted to growth in the chick-embryo by Koprowski & Cox in 1948.[10] By the 50th serial embryo-passage the strain was sufficiently avirulent to permit its use (now widespread) for canine immunization,[7, 8] but in the highly susceptible bovine it still produced occasional rabies encephalitis. Between the 176th and 182nd passage in embryos, the strain lost its ability to produce fatal encephalitis in cerebrally inoculated adult mice although it retained its ability to cause lethal encephalitis in infant mice; concurrently it ceased to cause disease on extraneural inoculation of cattle.[9] Because of its minimal virulence for animals, this high egg passage (HEP) virus was chosen for experimental immunization of man.

Initial interest lay in the possible use of HEP Flury vaccine for safe and effective primary immunization in emergency or post-exposure situations. This interest originated from the generally recognized limitations of the current forms of Pasteur treatment—namely, not only that they may fail to protect persons subjected to severe exposure, who may develop disease after a very short incubation period; but also that their use is fraught with the hazard of serious demyelinating reactions.[12, 16] While the use of the newly available hyperimmune serum appears to offer promise of protection against disease with a short incubation period,[2, 6] nonetheless, some form of active immunization is still essential to accompany the serum, as well as for cases of less severe exposure in which serum is not indicated. To avoid the tissue-specific sensitization postulated as the cause of the demyelinating reactions, one possibility is the use of a vaccine free of central nervous system tissue, such as the HEP Flury vaccine.

Our initial studies, reported in 1954,[15] were restricted to the use of large intramuscular inocula for primary immunization (each dose consisted of 2.0 g of chick-embryo). From the theoretical standpoint, because of the direct correlation observed between total dosage and antibody response, the results suggested that, even though living, HEP Flury virus does not multiply in man. From the practical standpoint, while some confirmation was obtained as to primary safety, it was found that excessive amounts of embryo tissue (total doses of 12 g to 20 g) were necessary to ensure the desired uniformity of response. Subsequent work, reported elsewhere in preliminary fashion,[4, 5] has been directed, with only small success, towards increasing the concentration of viral antigen in the vaccine and, with somewhat greater success, towards more efficient utilization of the available antigen by better selection of route and intervals of inoculation. This latter effort has been extended, for comparative purposes and with particularly interesting results, to a few experiments with the Semple type of vaccine. Related to the problem of primary emergency immunization has been a study of the influence of hyperimmune serum on primary

active response. And finally, a fairly extensive study has been made of the use of HEP Flury vaccine to restimulate immunity induced either by the same vaccine or, in a much larger series of cases, by previous conventional Pasteur treatment. Although HEP Flury vaccine has not provided a complete answer to all the problems investigated, it is felt that the available results afford sufficient guidance as to the fundamental principles of human antirabies prophylaxis and that their detailed presentation at this time is therefore warranted.

Materials and Methods

Vaccines and serum

HEP Flury vaccines in all instances consisted of homogenized chick-embryo material infected with virus of the 187th to 202nd passage level and lyophilized as a 40% embryo suspension. Most of the lots were prepared in embryos inoculated after 7-8 days' incubation, harvested 10 days later, blendorized and partially clarified by coarse filtration through gauze. Because the residual particles tended to obstruct the small-gauge needles necessary for intradermal inoculation, certain lots were clarified by horizontal centrifugation at 2000 revolutions per minute for 20 minutes. Finally, in an effort to gain both increased viral concentration and a more readily homogenized preparation, a few lots were prepared in embryos inoculated after 3 days' incubation and clarified by filtration only.

Other vaccines employed in small comparative groups were of commercial origin and included the Semple, Fermi and Harris types. In the case of persons recruited with a history of previous Pasteur treatment the type of vaccine employed was not always ascertainable.

Hyperimmune serum was administered in doses governed roughly by body-weight, averaging 5000 International Units [19] in 50 ml. The product used was that produced by Laboratorios Sclavo in Siena, Italy, and partially purified and distributed under licence by Lederle Laboratories.

Volunteers

The work first reported [15] was done in a small initial part on members of the Tulane Medical School staff and in larger part on adult male volunteers of both white and coloured races who were inmates of the Mississippi State Prison at Parchman, Miss. Also included in the first studies were nine persons, adults of both sexes, in the Pasteur Clinic of the Charity Hospital of New Orleans who were undergoing antirabies prophylaxis after dog bites. Subsequent work on primary immunization and on the recall of immunity induced by HEP Flury vaccine was done largely on prisoner inmates at Parchman or, in larger part, on inmates of the Louisiana State Penitentiary at Angola, La. In all the prison work, volunteers were recruited without proffered inducement of any kind, and only

after being fully informed of the objectives of the study and of the hazards and inconveniences which might be incurred by their participation. Before being accepted, each prisoner-volunteer was evaluated medically on the basis of a brief pertinent history and physical inspection. Several additional groups, largely comprising veterinarians and public health workers, were recruited at veterinary medical meetings in California, in several southern States, and among the participants in rabies seminars conducted by WHO in Muguga, Kenya, and in Caracas, Venezuela. These included many individuals who had previously received one or more courses of Pasteur treatment.

Observation of the volunteers

In the initial work, volunteers were observed daily for three weeks by interrogation as to subjective complaints, the taking of oral temperatures and inspection of the inoculation site. Subsequently, this was reduced to simple interrogation, often on a retrospective basis (outside the prisons). Otherwise, observation consisted principally of collection of blood specimens at stated intervals for laboratory study.

Inoculations

Large doses of HEP Flury vaccine (0.33-2.0 g or more) were given as 67% suspensions intramuscularly into either the upper arm (deltoid muscle) or the buttocks. The larger volumes (3.0 ml or, in a few instances, 4.5 ml) were given originally in a single site but in later work were divided between two or three sites. Single-dose syringes and 21-gauge (0.80 mm) needles were employed. Smaller doses (containing from 0.04 g to 0.16 g) were given in volumes of 0.1 ml or 0.2 ml per site, intradermally, either into the skin of the palmar surface of the forearm or over the deltoid insertion in the upper arm.

The other vaccines were given subcutaneously in doses of 0.5 ml (Semple and Harris) or 2.0 ml (Fermi).

The hyperimmune serum was given only after careful interrogation as to previous serum treatment and other allergic history and after skin testing (0.1 ml of 1:100 dilution, intradermally). The serum was usually given intramuscularly in multiple sites in the buttocks in a total volume of 50 ml.

Serological procedures

Tests were limited entirely to the demonstration of neutralizing antibody. The usual procedure was to screen the sera in a single dilution (1:4 final) shortly after collection and to do indicated quantitative tests at a later date. Unfortunately, in a significant number of instances the quantitative tests

were too long deferred and the sera, stored at 4°C in screw-cap vials, had become unusable because of mould contaminations.

All testing was done using the CVS rabbit-fixed strain of rabies virus. Virus and serum dilutions were made in a diluent of 2% normal guinea-pig serum in normal saline. In New Orleans, where most of the serological work was done, all tests were made with a constant amount of virus (approximately 100 LD_{50} per mouse inoculum) mixed with an aliquot of appropriately diluted serum. Such mixtures were kept for 1 hour at 37°C and a second hour at 4°C before inoculating 6 mice intracerebrally with serum-dilution and virus mixture. Titrations were based in most cases on testing sera in serial twofold dilutions, 50% end-points being calculated by the Reed & Muench method.

At the Lederle Laboratories, in Pearl River, N.Y., where some original testing and much of the crucial retesting were done, two basically different methods were employed. The first was similar to that described above except that the constant amount of virus was reduced to approximately 30 LD_{50}. The second method, referred to as the long incubation test, involves mixing 9 parts of undiluted serum (including a normal or pre-inoculation specimen as a control) with 1 part of a virus suspension titering 3.0 or more logs. Originally this mixture was held at 4°C for 6 days ; currently it is held at 37°C for 3 hours. For screening procedures two or three dilutions (undiluted, 1 : 5, 1 : 25 or 1 : 100) are made of the mixture and inoculated intracerebrally into mice. A significantly reduced mortality in the mice receiving the post-inoculation serum, as compared with that of mice given the control mixture, is interpreted as a positive test. For quantitative purposes, test and control mixtures were fully titrated in 5 mice with fivefold dilution. The result is expressed as the neutralization index (NI) or log difference between the titres of the control and test serum mixtures. NI's of less than 1.0 are not regarded as significant.

Observations

Primary safety of HEP Flury vaccine

The number of persons who have received primary courses of one inoculum or more of HEP Flury vaccine has now reached such a figure that reasonably firm statements can be made as to primary safety. Tables I and III show a total of 567 volunteers. To this may be added 6 persons, each given a single small intradermal inoculum, who were not included in the tables, 21 persons given hyperimmune serum plus HEP Flury vaccine, and 35 persons (cancer patients) given low passage (50th) Flury virus. An additional 30 persons received single inocula of 2.0 g in the recently reported comparative studies conducted by WHO [1] and 35 received a 3-dose (intradermal) course in Caracas, Venezuela. An additional 64

booster inocula (see Table VII) have been given to persons primarily immunized with HEP Flury vaccine, 23 subsequent boosters have been given to some of the foregoing, and 136 boosters were administered to volunteers with a history of previous Pasteur treatment. The total number of primary courses is thus 694 and that of the boosters is 223.

With two exceptions no significant systemic reactions have been observed. The two exceptions represented instances of immediate and alarming anaphylactoid reactions following injection into a single intramuscular site of 2.0 g of embryo tissue in a volume of 3.0 ml during primary immunization. Fortunately, prompt use of adrenalin resulted in rapid recovery of both volunteers. Since neither individual subsequently manifested a positive skin test to the vaccine, true allergic reactions seemed to be ruled out. It is believed that the excessive volume inoculated into a single site caused tissue disruption and entrance of finely particulate embryo material directly into the blood stream. Because of these episodes, similar large intramuscular inocula have been distributed into two or more sites. In the total of 200 booster inocula given, no instances of allergic sensitivity were encountered.

Except as mentioned above, the only systemic manifestations were occasional instances of transient fever, malaise, and bodily aching; however, when controls were given uninfected embryo material such minor reactions were equally frequent. In spite of the large inocula often employed, no clear instances of " serum sickness " have been observed which could be connected with the use of the vaccine.

Local reactions at the sites of intramuscular inocula were limited to moderate tenderness persisting for no more than two days. At intradermal sites somewhat more significant reactions may be provoked. Because of the considerable amount of embryo protein inoculated, the resulting nodule regresses slowly. It usually is surrounded by a zone of erythema 7-10 cm in diameter, which lasts for a few days. Occasionally a much larger zone of erythema is observed, accompanied by considerable swelling. Local tenderness is the rule, but mild in degree. Some local pruritus has also been reported. Regional lymphadenopathy has been noted in about 25% of cases. Not infrequently the persisting nodule undergoes sterile suppuration, ulcerates and discharges a small amount of pus two weeks or more after inoculation.

In summary, no reactions attributable to the HEP Flury virus *per se* have been observed. Further, the embryo protein inoculated (often in large amount) seems to be so weakly antigenic that it induces neither " serum sickness " nor subsequently developing sensitivity. The frequent inclusion in the vaccine of small amounts of penicillin, however, warrants a word of caution in respect of persons with known sensitivity to that antibiotic. Local reactions to intradermal inocula are common, but only infrequently are they of truly annoying proportions.

Primary immunization: Summary of previously reported observations

Because much of the work in previous publications [4, 5, 15] has been reported in preliminary fashion, and as considerable additional data have been collected as the result of quantitative retesting of numerous sera, it seems desirable to make a final and definitive report on this earlier work.

For obvious reasons, evaluation of methods of primary immunization must depend on observations as to the development of neutralizing antibody. Since primary immunization is usually practised on an emergency or post-exposure basis, we are interested not only in the ultimate level of antibody induced but also in the time of its appearance. In Table I are summarized observations as to the time of appearance of neutralizing antibody in 54 persons given various courses of Harris- or Semple-type vaccine and in 247 persons given HEP Flury vaccine. In terms of ultimate response, the record with Harris and Semple vaccines is clearly gratifying, only a single failure being recorded. Particular attention should be given to the 19 persons receiving only 4 doses of Semple vaccine, one dose every 5 days. While essentially all of the 38 persons receiving the 4-, 12- or 14-dose schedules of the Semple or Harris vaccines had antibody by the 15th day after the first inoculation, in only 10 (26%) was antibody demonstrated in the 10th-day serum.

HEP Flury vaccine was given in courses varying in respect of the size of the individual inocula, the number and spacing, and the route of inoculation. If one looks first at the results obtained with the 2.0-g inocula, three points of interest become evident. First, in the case of courses completed within a 15-day period (inocula closely spaced), there is a direct relation between the total amount of embryo material inoculated and the over-all percentage responding—from 17% after 2.0 g to 100% after 20 g. This observation is the fundamental reason for believing that HEP Flury virus does not multiply in man. Second, if one considers courses of two inocula only, with a total dose of 4.0 g, there is a clear relation between spacing of the inocula and response. Unfortunately, the 30-day interval associated with 100% response is too long for purposes of emergency immunization. Third, of the 43 persons actually responding to total inocula of 6.0 g to 20.0 g, 23, or more than one half, had antibody by the 10th day.

A somewhat greater number of observations has been made with smaller inocula consisting either of 0.33 g per intramuscular site or 0.04-0.08 g per intradermal site. In some courses multiple sites were inoculated simultaneously. As with the larger doses, single inocula (not shown) and 2 inocula separated by no more than 15 days were relatively ineffective. However, it is noteworthy that 2 small intradermal inocula were at least as effective as 2 medium or large intramuscular inocula, thus suggesting the greater efficiency of the intradermal route. At the other extreme 6-8 inocula at 3-day or 2-day intervals gave only 78% response when the

TABLE I. TIME OF APPEARANCE OF ANTIBODIES FOLLOWING PRIMARY IMMUNIZATION WITH HARRIS, SEMPLE AND HEP FLURY VACCINE: SUMMARY OF PREVIOUS OBSERVATIONS

Inoculation data					Number of persons inoculated	Number of persons with antibody[a] on indicated day after first inoculation						Total percentage responding[b]
vaccine	single dose	route	number of doses	intervals (days)		0	10	15	20	30	60	
Harris	0.5 ml	SC	14	1	9	0	2	9	9	9	9	100
Semple	0.5 ml	SC	12	1	10[c]	0	2	8	9	9	9	90
			4	5	19	0	6	17	19	19	19	100
			2	10	13	0	1	12	13	12	12	100
			2	15	3	0	—	2	3	3	2	100
HEP Flury	2.0 g in 3 ml (in 1, 2 or 3 sites)	IM	3-4	1 or 3	42	0	9	12	13	17	17	48
			6	1-7	14	0	7	8	8	9	12	93
			5, 10	1 or 2	10	0	7	7	7	8	9	100
	2.0 g in 3 ml	IM	1	—	12	0	—	0	—	2	2	17
			2	3	14	0	—	0	—	6	6	43
				10-20	18	0	—	7	10	10	10	55 (61)
				30	6	0	—	—	—	2	6	100
	0.33 g in 0.5 ml (in 1 or 2 sites)	IM	2	10-15	8	0	—	2	2	2	3	37 (67)
				30	4	0	—	—	—	1	3	75 (100)
			3-4	5	18	0	5	9	10	12	13	78 (100)
			8	2	4	0	0	3	4	4	4	100
	0.08 g (in 2 sites) + 0.33 g (in 2 sites)	ID + IM	4	5	8	0	5	7	7	8	8	100
	0.04-0.16 g in 0.1 or 0.2 ml (in 1 or 2 sites)	ID	2	5-10	14	0	2	7	6	4/7[d]	6/7[d]	50
			3	5	16	0	6	10	11	12	11	88
			4	5	19	0	8	14	16	18	18	95
			6-8	3-2	9	0	0	4	5	7	3/4[d]	78
HEP Flury, low titre lot	0.04 g	ID	6	3	6	0	0	0	3	3	—	67 (100)
	0.04 g (in 4 sites)	ID	2	10	14	0	1	1	2	6	1/6[d]	50
	0.04 g (in 4 sites)	ID	4	5	11	0	1	3	4	4	4	54

[a] Antibody as indicated by survival of 3 or more of 6 mice inoculated with mixture of 1 : 4 final dilution of serum and 100 LD_{50} of virus.

[b] Based on presence of significant neutralization in at least one serum specimen; apparent discrepancies between percentage shown and maximum number positive on any one day are due to individual variations in time of relatively transient low level responses. Figures in parentheses are based on retesting sera negative in the conventional test by means of the long incubation test.

[c] Sera from these ten persons were obtained through the courtesy of Dr M. M. Kaplan, Veterinary Public Health Section, World Health Organization.

[d] When less than the full number of sera were available for testing, the results are shown as the ratio of those positive to those tested.

intradermal route was employed, and 100% in four men inoculated intramuscularly, but this is obviously too small a group to be of significance. Of the combinations tested more adequately, the most promising utilized 3 or 4 doses with a 5-day interval, perhaps the best being 4 intradermal doses which immunized 18 of 19 volunteers (serum from the "non-responder" was not retested in the long incubation test) or—but this was not adequately tested—a course of simultaneous intradermal and intramuscular inocula on four occasions which immunized quite promptly all of the 8 volunteers. The last three lines of the table constitute apparent exceptions to the foregoing statements and illustrate the inferior results associated with a vaccine with low antigenic potency. Altogether, of the 61 persons given the most satisfactory courses (3 or 4 inocula with 5-day spacing) 24 (37%) developed antibody by the 10th day.

The data concerning level of antibody developed are presented in Table II. These refer to fewer individuals than do the data in Table I because of the elimination of persons receiving some of the courses of lesser interest, and, unfortunately, because not a few sera had become unsuited for titration as the result of mould contamination. It is evident at once that, although there is considerable overlapping, the titres engendered by courses of Semple or Harris vaccine tend to exceed those induced by the most promising courses of HEP Flury vaccine. Two additional points merit special comment. First is the fact that the 4-dose course of Semple vaccine stimulated antibody titres which at least equalled those following the 14-dose course of Harris vaccine. Second, 4 intradermal doses of HEP Flury vaccine yielded titres which at 60 days were definitely higher than those following 3 intradermal doses and were at least equivalent to those following any of the courses involving larger intramuscular doses.

In summary, in terms of both total percentage responding and level of antibody stimulated, no course of HEP Flury vaccine achieved results equivalent to those following 4 or more doses of Harris or Semple vaccine. The most efficient use of HEP Flury vaccine was by intradermal administration and the most promising course involved the use of the 5-day interval with 4 doses given intradermally, possibly accompanied by simultaneous intramuscular inocula. However, in terms of rapidity of response, as measured by appearance of antibody by the 10th day after first inoculation, the best course of HEP Flury vaccine proved possibly superior to those of Harris or Semple vaccine.

Primary immunization: New observations

The most recent work relating to primary immunization has centred on efforts to improve the antigenic potency or the ease of administration of the HEP Flury vaccine and on a study of the possible influence of hyperimmune serum upon response to active immunization.

TABLE II. OBSERVATIONS ON LEVEL OF NEUTRALIZING ANTIBODY FOLLOWING PRIMARY IMMUNIZATION WITH HARRIS, SEMPLE AND HEP FLURY VACCINE: SUMMARY OF WORK DONE PREVIOUSLY

Type of vaccine	Total dose	Number of inocula	Interval (days)	Total number of persons	Days after first inoculation	Number of persons with antibody titre in indicated range of 1:					
						<4	4-7	8-31	32-127	128-511	512+
Harris	7.0 ml SC	14	Daily	9	15			2	4	3	
				9	30				2	3	4
				9	60				4	5	
Semple	1.0 ml SC	2	10-15	13	15	1	2	3	6	1	
				16	30	1	2	4	6	3	
				16	60	2[a]	3	5	4	2	
	2.0 ml SC	4	5	19	15	2		1	7	7	2
				19	30			1	4	9	5
				19	60			1	8	10	
HEP Flury	2.0 g IM	1	—	15	15	15					
				15	30	14	1				
				15	60	13	2				
	4.0 g IM	2	3	14	15	14					
				14	30	9	5				
				14	60	9	5				
			10	8	15	6	1	1			
				8	30	4	3	1			
				8	60	3	4		1		
			15-20	11	15	6	4		1		
				11	30	7		3		1	
				11	60	6	2	2		1	
			30	5	30	4	1				
				5	60		2	1	2		
	6.0 g IM	3	1, 3	30	15	22	6	2			
				30	30	25	3	1	1		
				30	60	21	7		2		
	8.0 g IM	4	3	14	15	9	5				
				14	30	8	6				
				14	60	9	3	2			
	12.0 g IM	2, 3, 6	2, 7	14	15	8	1	3	2		
				14	30	6	1	4	3		
				14	60	2		6	6		
	20.0 g IM	5, 10	1, 2	6	15	2	1		3		
				6	30			3	3		
				6	60			3	3		

TABLE II. OBSERVATIONS ON LEVEL OF NEUTRALIZING ANTIBODY FOLLOWING PRIMARY IMMUNIZATION WITH HARRIS, SEMPLE AND HEP FLURY VACCINE: SUMMARY OF WORK DONE PREVIOUSLY (continued)

Type of vaccine	Total dose	Number of inocula	Interval (days)	Total number of persons	Days after first inoculation	Number of persons with antibody titre in indicated range of 1:					
						<4	4-7	8-31	32-127	128-511	512+
HEP Flury	0.66 g IM	2	10	4	15	2	1	1			
				4	30	2	2				
				4	60		4				
			15	4	15	4					
				4	30	4					
				4	60	3	1				
			30	4	30	4					
				4	60	1	2	1			
	0.99 g IM	3	5	4	15		3	1			
				4	30		3	1			
				4	60		4				
	1.32 g or 2.64 g IM	4	5	12	15	6	3	3			
				12	30	2	5	3	1	1	
				12	60	2	4	5	1		
	2.64 g IM	8	3	4	20		2	2			
				4	30		2	2			
				4	60		2	2			
HEP Flury	2.64 g IM + 0.32 g ID	4	5	8	15	1	1	2	3	1	
				8	30			3	5		
				8	60			4	4		
HEP Flury	0.04 g ID	1	—	7	15-60	7					
	0.08 g ID	2	5, 10	14	15	6	7	1			
				14	30	10	2	2			
				14	60	8	5	1			
	0.10-0.96 g ID	3	5	45	15	19	16	8		2	
				45	30	11	14	13	5	2	
				45	60	15	10	13	7		
	0.16-0.64 g ID	4	5	19	15	5	7	6	1		
				19	30	1	8	6	2	2	
				19	60	3	6	4	2	3	1
	0.24 g ID	6	3	5	15	3	2				
				5	30	1	2	1	1		
	0.32 g or 1.28 g ID	8	2	4	15	2		2			
				4	30	1	1	2			
				4	60	1	1	2			

[a] One serum negative by conventional test but positive by long incubation test.

Efforts to increase the antigenic potency of the vaccine and to eliminate extraneous embryo material took several forms. Efforts were made in both New Orleans and Pearl River to produce viral antigen in chick-embryo tissue-culture. Virus yields barely achieved titres of 2.0 logs and were considered inadequate for use. Efforts were also made in Pearl River to purify the virus contained in the usual embryo homogenate both by alcohol precipitation and by differential centrifugation. In neither instance was active material recovered. Finally, in an effort similar to but independent of that reported recently by Yoshino and co-workers,[20] vaccine was produced in embryos inoculated after 3 days' incubation and harvested 5 days later. Infectivity titres consistently exceeded 5 logs and several lots of vaccine were prepared with clarification by coarse filtration only.

TABLE III. NEW OBSERVATIONS WITH HEP FLURY VACCINE: TIME OF APPEARANCE OF NEUTRALIZING ANTIBODY

Experiment	Type of vaccine	Embryo concentration (%)	ID inoculum (g)	Number of inocula	Interval (days)	Number of persons inoculated	Number of persons with antibody [a] on day indicated after first inoculum						Total percentage responding [b]
							0	10	15	20	30	60	
A	Centrifuged	80	2 · 0.16	3	5	9	0	5	6	8	7	6	89 (100)
		40	2 · 0.08	3	5	7	0	4	5	7	6	6	100
	Filtered	40	2 · 0.08	3	5	9	0	4	6	8	8	6	100
		12.5	2 · 0.03	3	5	8	0	4	4	5	4/7 [c]	4/5 [c]	87
B	Filtered	40	Initially 2 · 0.08, then 1 · 0.08	3	5	12	0	5	—	—	7	—	60 (92)
C¹ [d]	Filtered	40	0.08	3	5	116	0	—	—	—	62 (76)		53 (66)
C² [d]	Filtered	40	0.08	3	5	29	7	—	—	—	17 (21)		60 (72)
D	Young embryo	70	0.14	3	5	9	0	—	—	—	1	1	11
E	Young embryo	70	0.14	3	5	11	1 [e]	1	1	1	1	—	0
		70	0.14	4	5	10	0	0	0	0	0	—	0

[a] Antibody as indicated in footnote *a* to Table I, or, in Experiments C¹ and C², on the basis of the long incubation est.

[b] As in footnote *b* to Table I, except in Experiments C¹ and C², in which only the long incubation test was performed; the figures in parentheses are based on inclusion of doubtful or inconclusive responders.

[c] As in footnote *d* to Table I.

[d] Experiment C¹ involves California veterinarians with definite history of not having had prior Pasteur treatment; C² involves persons for whom no history is available and obviously includes some who have had prior treatment.

[e] Serum from one volunteer neutralized virus to same partial degree (4/6) in all specimens including pre-vaccination.

In addition to increased infectivity, this young-embryo vaccine was more readily homogenized and could be inoculated easily in 70% concentration through small-gauge needles. As indicated in Table III (Experiments D and E) 30 volunteers were given 3 or 4 doses of this vaccine from two lots. The very disappointing, nearly complete failure to respond remains to be explained.

Experiments A and B, reported in Tables III and IV, and Experiments C^1 and C^2 reported only in Table III, proved more interesting. Experiment B represented merely a minor variation of previous schedules, i.e., an initial intradermal dose of the usual filtered vaccine consisting of 0.08 g given in each of two sites followed by 0.08 g per single site on two occasions at 5-day intervals. Experiments C^1 and C^2 represented an expansion with a new lot of vaccine of an already tested 3-dose course (0.08 g per dose intradermally with 5-day intervals). The results in both instances were less satisfactory in terms of both total percentage responding and antibody levels achieved than were those of comparable earlier experiments. In

TABLE IV. NEW OBSERVATIONS WITH HEP FLURY VACCINE: TITRES OF NEUTRALIZING ANTIBODY DEVELOPED IN EXPERIMENTS A AND B OF TABLE III

Experiment	Inoculation data (ID)	Type of vaccine	Embryo concentration (%)	Number of persons	Days after first inoculation	Number of persons with antibody titre in indicated range of 1:					
						<4	4-7	8-31	32-127	128-511	512+
A	3 doses of 0.08 g in 2 sites at 5-day intervals	Centrifuged	80	9	15	3	3	2		1	
				9	30	2	2	4		1	
				9	60	3	1	4	1		
			40	7	15	2	4			1	
				7	30	1	2	3		1	
				7	60	2	1	3	1		
		Filtered	40	9	15	3	5	1			
				9	30	1	6	2			
				9	60	3	4	2			
			12.5	8	15	4	2	2			
				8	30	3	2	2	1		
				8	60	2	3	2	1		
B	Initially 0.08 g in 2 sites, then 2 doses of 0.08 g at 5-day interval	Filtered	40	12	10	10	1	1			
				12	30	6	4	1	1		

Experiment A a single original lot of vaccine was divided into two parts before lyophilization; one part was clarified by centrifugation (see "Materials and Methods" above). The centrifuged material was so free of grossly particulate material that it could pass readily through 25-gauge (0.50 mm) needles in 80% concentration. The data for Experiment A on time of appearance of antibody, total percentage responding and antibody levels

TABLE V. OBSERVATIONS ON THE INFLUENCE OF HYPERIMMUNE SERUM UPON RESPONSE TO ACTIVE IMMUNIZATION

Course of active immunization	Day on which serum given	Number of persons	Days after first inoculation	Number of persons with antibody titres in indicated range of 1:				Evaluation ratio [a]
				<4	4-7	8-31	32+	
HEP Flury, ID: 6×0.04 g at 3-day intervals	0	6	30	4	1	1		5/6 [b] (6/6)
			60	6				
	1	7	30	3	2	2		5/7 (6/7)
			60	7				
	not given	5	30	1	2	1	1	4/5 (5/5)
			60	—[c]	—[c]	—[c]	—[c]	
HEP Flury, ID: 3×0.16 g at 5-day intervals	0	8	30	2	3	2	1	6/8
			60	5	2		1	
	not given	9	30	1	6	2		9/9 [b]
			60	3	5	1		
Fermi, SC: 3×2.0 ml at 5-day intervals	0	11	42	7	3	1		4/11 [d]
			60	9	1	1		
	0,5	9	42	3	3	3		4/9 [e]
			60	5	2	2		
	not given	12	42	6	4	2		6/12
			60	8	1	3		

[a] Ratio of persons responding to persons vaccinated; figures in parentheses are based on results with long incubation test.

[b] The apparent discrepancy between evaluation ratio and data as to antibody titre is explained by the inclusion in the column headed "< 4" of sera with very low antibody titre.

[c] Sera not usable when titrations were to be performed.

[d] Two individuals considered to have responded still had demonstrable antibody in their 60-day sera but in titre less than 1 : 4.

[e] In at least 2 individuals in this group antibody present at 42 days is believed to have been derived from hyperimmune serum and not as a result of active response; in both cases the 60-day sera were completely devoid of antibody in the lowest dilution tested (final 1 : 4).

developing are presented in Tables III and IV for groups of 7-9 volunteers given a course of 3 inoculations at 5-day intervals with materials of each type in two different concentrations. Comparison of the groups receiving 40% material indicates that centrifugation did not diminish the antigenic potency of the vaccine. It is also evident that a twofold or threefold increase or decrease in concentration of the vaccine did not greatly influence the response.

In the report on comparative tests conducted under WHO auspices,[1] evidence was given suggesting that the presence of passively acquired antibody from hyperimmune serum may exert a slight suppressive effect upon active immune response. Three additional experiments to test this point have been carried out. Since antibody carried over from the hyperimmune serum may persist for as long as three or four weeks, attention has been limited to antibody present 30 or more days after the first inoculation of vaccine. The results are given in Table V. In the first experiment, which was done with HEP Flury vaccine and in which the control data are unfortunately incomplete, an effort was made to see whether by delaying administration of serum one day the suppressive effect could be reduced. While the over-all results suggest that some slight suppression occurred, there is no clear difference related to the time of giving the antiserum. The second experiment, also done with HEP Flury vaccine, was a simple effort to measure more precisely the suppressive effect. The results are more definite in demonstrating such an effect in that of the 8 receiving serum plus vaccine, 2 failed to manifest active response and 5 had no demonstrated antibody at 60 days, whereas of 9 given only vaccine all responded and all but 3 still had antibody after 60 days. The third experiment was carried out as part of a new set of comparative tests sponsored by WHO with the intent of studying the practical importance of the suppressive effect on what was hoped would be a usable but abbreviated course of potent phenolized vaccine. Unfortunately for the original objective, the potency of the vaccine was extremely low. However, this very marginal potency was thought to offer a possibly more crucial test of the suppressive phenomenon itself.

In this experiment all men received a 3-dose course of vaccine at intervals of 5 days; one group received no serum; another received serum on the same day as the first inoculation of vaccine; and the third received a second dose of serum 5 days later, as well as on the same day as the first inoculation of vaccine. The results, also in Table V, are somewhat difficult to interpret with respect to the group given 2 doses of serum, since serum-derived antibody apparently persisted in a few instances for as long as the 42nd day. In all, the best response was that of the group which received only vaccine but, once again, the differences are not impressive. These three experiments, therefore, lend support to the idea that, while the presence of serum-derived antibody may exert some depressive influence on active antibody response, such influence is slight and probably would be of no practical importance with respect to a really adequate course of active immunization.

TABLE VI. ANTIBODY RESPONSES OF PERSONS EXPERIMENTALLY IMMUNIZED AND GIVEN SINGLE BOOSTER INOCULUM OF HEP FLURY VACCINE

Individual	Primary course				Maximum immune response	Interval since primary	HEP booster inoculum	Response to booster [a] on day					Evaluation [b]
	vaccine	single dose	number of doses	interval (days)				0	10	15	30	60	
V.H.	SE	0.5 ml IM	2	10	16	5 months	0.04 g ID	5/6	6/6 [c]	6/6 [c]	6/6 [c]	6/6 [c]	R
W.M.					>32	,,	0.33 g IM	16	>24	>32	32	>32	R
A.L.	HEP Flury	2.0 g IM	2	10	>4	5 months	0.04 g ID	0	4	16	8	16	R
R.W.				15	2/6 0.85 NI [e]	,,	,,	0	—	1/6	1/6 1.45 NI [e]	1/6	R
C.W.				30	>4	,,	,,	0	—	—	6/6 [c]	6/6 [c]	R
R.B.				10	32	,,	0.33 g IM	6/6 [c]	6/6 [c]	6/6 [c]	6/6 [c]	6/6 [c]	R ?
C.L.				10	3/6 0.25 NI [e]	,,	,,	12	6/6 [c]	6/6 [c]	6/6 [c] 2.10 NI [e]	6/6 [c]	R ?
T.P.				30	128	,,	,,	2/6	6/6 [c]	6/6 [c]	6/6 [c]	6/6 [c]	R
J.F.			1	—	0 0 NI [e]	12 months	2.0 g IM	0	2/6	0 1.25 NI [e]	—	—	R ?
D.C.				—	1/6	,,	,,	0	28	48	—	—	R
D.N.				—	1/6 0.5 NI [e]	,,	,,	0	64	64 >2.80 NI [e]	—	—	R
A.M.				—	0	,,	,,	0	64	32	—	—	R
Wa.W.				—	0 0 NI [e]	,,	2.0 g + 2.0 g [d]	0	—	—	>256	>256	R
C.M.				—	2/6	,,	2.0 g + 2.0 g [d]	0	—	—	5/6	64	R

L.M.			2	3	2/5 0 NI [e]	12 months	2.0 g IM	0	1/6 1.95 NI [e]	—	—	—	R
J.S.				3	2/6	,,	,,	2/6	96	96	—	—	R
C.B.				15	6/6	,,	,,	0	6/6 [c]	6/6 [c]	5/6 [c]	—	R
C.F.				15	1/6	,,	,,	0	6/6 [c]	6/6 [c]	2/6	—	R
G.A.				20	2/6	,,	0.33 g IM	0	6/6 [c]	6/6 [c]	6/6 [c]	—	R
W.Mc.				20	1/6	,,	0.04 g ID	0	32	32	8	8	R
H.J.			3	1	2/6	12 months	2.0 g IM	0	3/6	2/6 2.0 NI [e]	—	—	R
K.J.				1	2/6	,,	,,	1/6	24	32	—	—	R
G.G.				1	4/6	,,	,,	1/5	>60	>60	—	—	R
Wi.W.				1	8	,,	,,	16	>1024	>1024	—	—	R
A.T.				3	32	,,	,,	24	>1024	>1024	—	—	R
R.H.				3	1/6	,,	,,	8	48	48	—	—	R
H.M.				3	0 0 NI [e]	,,	,,	1/6	1/6	0/6 0 NI [e]	—	—	N
J.H.			4	3	16	12 months	2.0 g IM	24	>1024	>1024	—	—	R
L.G.				3	6/6	,,	,,	32	>128	6/6 [c]	—	—	R
T.H.				3	4/6	,,	,,	0	>512	400	—	—	R
L.J.			5	2	16	12 months	2.0 g IM	1/6	—	>256	—	—	R

[a] The response is indicated variously as follows: (1) as protection ratio of 1 : 4 final serum dilution tested with about 100 LD_{50} of virus, i.e., number of mice surviving over number inoculated; (2) as reciprocal of 50% serum dilution end-point as determined using 100 LD_{50} of virus; or (3) as neutralization index (log virus neutralized) by undiluted serum in long incubation test.

[b] Evaluation: R = response to booster; R? = status uncertain; N = no response.

[c] Specimen could not be retested to determine final titre because of inadequate volume or unsatisfactory state after prolonged storage.

[d] 2 booster doses, 20 days apart (erroneously included in new trial group).

[e] NI = Neutralization index, long incubation test.

TABLE VI. ANTIBODY RESPONSES OF PERSONS EXPERIMENTALLY IMMUNIZED AND GIVEN SINGLE BOOSTER INOCULUM OF HEP FLURY VACCINE (continued)

Individual	Primary course				Maximum immune response	Interval since primary	HEP booster inoculum	Response to booster [a] on day					Evaluation [b]
	vaccine	single dose	number of doses	interval (days)				0	10	15	30	60	
W.Wh.	HEP Flury	2.0 g IM	2×3	7	16	12 months	0.001 g ID	4/6	16	6/6	16	24	R
B.P.			2	3	2/6 0 NI [e]	24 months	0.04 g ID	0	0	0	0 0.55 NI [e]	—	N
C.K.				3	1/6	,,	,,	0	2/6	8	12	96	R
R.R.				3	8	,,	0.08 g ID	2/6	—	192	48	12	R
A.W.			3	3	3/6	24 months	2.0 g IM	0	6/6 [c]	6/6 [c]	6/6 [c]	3/6	R
T.D.			4	3	6/6	24 months	0.04 g ID	2/6	6/6 [c]	6/6 [c]	48	—	R
H.S.				3	3/6	,,	,,	0	>32 [c]	>32 [c]	—	>32 [c]	R
A.K.				3	6/6	,,	,,	0	32	16	32	—	R
R.P.				3	3/6	,,	,,	1/6	>32 [c]	32	16	32	R
J.G.			5	2	64	24 months	0.04 g ID	6/6 [c]	6/6 [c]	6/6 [c]	6/6 [c]	—	R?
W.O.			2×3	7	64	,,	,,	16	6/6 [c]	6/6 [c]	128	—	R
L.Y.			2	3	4/6	,,	0.33 g IM	1/6	>16	16	32	16	R
G.R.			10	1	96	34 months	0.08 g ID	6	—	>128	>128	—	R
C.Wh.			6	2	64	43 months	0.16 g ID	8	—	—	>32	—	R
J.Mc.			3	1	0 (P-LT) [f]	48 months	0.16 g ID	0	—	—	0	—	N

J.F.	HEP Flury	0.33 g IM	2	10	3/6	5 months	0.04 g ID	0	6/6 [c]	6/6 [c]	6/6 [c]	4/5 [c]	R
J.J.			2	10	5/6	,,	,,	0	—	256	—	—	R
O.H.			2	10	3/6	,,	,,	0	3/5	—	5/6 [c]	2/6	R
H.D.			4	5	3/6	,,	,,	0	6/6 [c]	6/6 [c]	4/5 [c]	6/6 [c]	R
R.F.			2	15	3/6	,,	0.33 g IM	0	—	>32	—	—	R
G.J.			2	15	4/6	,,	,,	0	6/6 [c]	6/6 [c]	6/6 [c]	6/6 [c]	R
A.T.			4	5	6	,,	,,	NS [g]	6/6 [c]	6/6 [c]	6/6 [c]	6/6 [c]	R ?
W.K.			2	15	2/6	,,	2.0 g IM	0	6/6 [c]	6/6 [c]	6/6 [c]	6/6 [c]	R
H.R.			4	5	5/6	,,	,,	0	6/6 [c]	—	6/6 [c]	6/6 [c]	R
Jo.J.			4	5	6/6	,,	,,	0	5/6 [c]	6/6 [c]	6/6 [c]	6/6 [c]	R
A.P.	HEP Flury	0.04 g ID	4	5	>16	5 months	0.04 g ID	3/6	5/6 [c]	6/6 [c]	6/6 [c]	5/6 [c]	R
L.R.			4	5	16	,,	,,	0	6/6 [c]	6/6 [c]	6/6 [c]	6/6 [c]	R
C.E.			3	5	3/6	,,	0.33 g IM	0	6/6 [c]	6/6 [c]	6/6 [c]	6/6 [c]	R
W.S.			3	5	16	,,	,,	5/6 [c]	6/6 [c]	6/6 [c]	6/6 [c]	6/6 [c]	R?
M.B.			1	—	5/6	12 months	0.04 g ID	1/6	2/6	3/6	5/6	—	R
F.B.			2	5	3/6	,,	0.33 g IM	0	6/6	6/6	>32	—	R
W.C.			2	5	32	,,	,,	6/6 [c]	6/6 [c]	6/6 [c]	6/6 [c]	6/6 [c]	R?
W.B.			2	5	2/6	24 months	0.08 g ID	4/6	—	—	48	—	R

[a] The response is indicated variously as follows: (1) as protection ratio of 1 : 4 final serum dilution tested with about 100 LD_{50} of virus, i.e., number of mice surviving over number inoculated; (2) as reciprocal of 50% serum dilution end-point as determined using 100 LD_{50} of virus; or (3) as neutralization index (log virus neutralized) by undiluted serum in long incubation test.

[b] Evaluation: R = response to booster; R? = status uncertain; N = no response.

[c] Specimen could not be retested to determine final titre because of inadequate volume or unsatisfactory state after prolonged storage.

[e] NI = Neutralization index, long incubation test.

[f] P-LT = Positive in long incubation test.

[g] NS = No specimen.

TABLE VII. SUMMARY OF ANTIBODY RESPONSES OBSERVED IN EXPERIMENTALLY IMMUNIZED PERSONS GIVEN SINGLE BOOSTER INOCULA OF HEP FLURY VACCINE

Interval since primary	Booster HEP Flury	Primary course	Response to primary	Number boosted	Number responding	Range of maximum response titre
5 months	0.04 g ID	2×0.5 ml SE	yes	1	1	>4[a]
		2×2.0 g HEP	yes	3	3	1/6-16 (1 not titrated)
		2-4×0.33 g HEP	yes	4	4	>4[a]-256 (3 not titrated)
		4×0.04 g HEP	yes	2	2	>4[a]
	0.33 g IM	2×0.5 ml SE	yes	1	1	32
		2×2.0 g HEP	yes	3	1 (3)	>4[a]
		2-4×0.33 g HEP	yes	3	2 (3)	>4[a]->32 (2 not titrated)
		3×0.04 g HEP	yes	2	1 (2)	>4[a]
	2.0 g IM	2-4×0.33 g HEP	yes	2	2	>4[a]
		2-4×0.33 g HEP	?	1	1	>4[a]
12 months	0.001 g ID	6×2.0 g HEP	yes	1	1	24
	0.04 g ID	2×2.0 g HEP	no	1	1	32
		0.04 g HEP	yes	1	1	5/6
	0.33 g IM	2×2.0 g HEP	?	1	1	>4[a]
		2×0.04 g HEP	yes	2	1 (2)	>4[a]->32 (1 not titrated)
	2.0 g IM	1-3×2.0 g HEP	no	4	2 (3)	1/6->256
		1-3×2.0 g HEP	?	9	9	1/6-96 (1 not titrated)
		1-5×2.0 g HEP	yes	8	8	>4[a]->1024 (1 not titrated)
24 months	0.04 g or 0.08 g ID	2-6×2.0 g HEP	?	4	3	>32[a]-96
		2-6×2.0 g HEP	yes	5	4 (5)	>4[a]-192 (1 not titrated)
	0.33 g IM	2×0.04 g HEP	?	1	1	48
		2×2.0 g HEP	yes	1	1	32
	2.0 g IM	3×2.0 g HEP	?	1	1	>4[a]
34-48 months	0.08 g or 0.16 g ID	6-10×2.0 g HEP	yes	2	2	>32->128
		3×2.0 g HEP	?	1	0	—
Grand total (5-48 months)	0.001-0.16 g ID	various	no	1	1	32
		various	?	5	3	>32[a]-96
		various	yes	19	18 (19)	1/6-256
	0.33 g IM	various	?	2	2	>4[a]
	0.33 g IM	various	yes	12	7 (12)	>4[a]-32
	2.0 g IM	various	no	4	2 (3)	1/6->256
		various	?	11	11	1/6-96
		various	yes	10	10	>4[a]->1024

[a] Specimen could not be retested to determine final titre because of inadequate volume or unsatisfactory state after prolonged storage.

Restimulation of immunity induced with HEP Flury vaccine

On the basis of the observations already reported,[4, 5] it has been suggested (*a*) that HEP Flury vaccine may condition a person to a later booster inoculum even when it fails to induce readily demonstrable immediate response; and (*b*) that a single inoculum of HEP Flury vaccine, even of small size, will elicit a booster response in persons previously conditioned either by the same vaccine or by previous Pasteur treatment. In Table VI are tabulated the data pertaining to 62 persons immunized primarily with Flury vaccine and to two immunized with Semple vaccine. With two exceptions in which two " booster " doses were inadvertently administered, 62 persons received single booster inocula of HEP Flury vaccine in doses ranging from 0.04 g (one with 0.001 g) to 2.0 g. In 3 instances it is reasonably certain that no response was elicited, these also being instances in which the primary course had induced no or an uncertain response. In 7 additional instances there is a likelihood that response was elicited but the data are not clear, often because spoilage of the serum prevented the crucial quantitative tests. In all other instances fairly clear-cut response was elicited, although again in too many cases the degree of response could not be determined because the sera were spoiled before titrations were attempted.

In Table VII data are summarized with respect to interval since the primary course and size of the booster inoculum. There are no obvious differences related to either variable. However, there is a suggested relation between the level of response to primary immunization and the maximum titres after the booster inoculum. This is shown in Table VIII for the 42 persons whose post-booster sera were at least partially titrated. This suggests that, to ensure good maintenance levels, the primary course itself should induce a readily demonstrable response.

A total of 18 persons received 2 or more booster inocula spread over intervals ranging from 5 months to 4 years. The results of this rather un-

TABLE VIII. CORRELATION OF MAXIMUM ANTIBODY TITRE AFTER HEP FLURY BOOSTER AND MAXIMUM TITRE FOLLOWING PRIMARY COURSE OF VACCINE

Maximum antibody titre after primary course (1:)	Total number of persons	Number of persons with maximum antibody titre (1:) after booster dose						
		0	Positive <4	4-7	8-31	32-127	128-511	512+
32+	5					2	2	1
8-31	5				1		2	2
4-7	6		1		1	2	2	
Positive <4	19	1	2	1		14	1	
0	7	3	1		1	1	1	

TABLE IX. RESULTS OBSERVED IN PERSONS RECEIVING

Volunteer	Primary course			First booster					Second	
	vaccine and schedule	date	response	interval	antibody level	inoculum (HEP Flury)	maximum response		interval	antibody level
							level	day		
M.A.	Pasteur	1928	?	24 years	0	2.0 g IM	1/6[a]	30	2 years	0[a]
T.L.	Pasteur	1934	?	18 years	8	2.0 g IM	>128	10	4 years	6
R.J.	Pasteur	1942	?	11 years	0	2.0 g IM	>256	30	3 years	24
B.B.	Pasteur	1952	?	2 years	8	2.0 g IM	104	20	5 months	6
R.B.	No history		?	?	64	2.0 g IM	>256	10	1 year	<16
L.S.	No history		?	?	5/6	2.0 g IM	>4[b]	30	2 years	32
W.W.	HEP 2.0 g	1953	0	1 year	1/6	2.0 g IM	>256	30	1 year	>4[b]
C.K.	HEP 4.0 g	1953	1/6	2 years	0	0.04 g ID	96	60	1 year	0
G.A.	HEP 4.0 g	1954	2/6	1 year	0	0.33 g IM	>4	10?	1 year	0
R.H.	HEP 6.0 g	1953	1/6	1 year	8	2.0 g IM	48	10	1 year	>4[b]
Wi.W.	HEP 6.0 g	1953	8	1 year	16	2.0 g IM	>1024	10	1 year	>4[b]
H.J.	HEP 6.0 g	1953	2/6	1 year	0	2.0 g IM	3/6	10	1 year	1/6
K.J.	HEP 6.0 g	1953	2/6	1 year	1/6	2.0 g IM	32	30	1 year	0
A.T.	HEP 6.0 g	1953	32	1 year	24	2.0 g IM	>1024	10	2 years	24
J.H.	HEP 8.0 g	1953	16	1 year	24	2.0 g IM	>1024	10	1 year	>4[b]
T.D.	HEP 8.0 g	1953	>4[b]	2 years	2/6	0.04 g ID	>32	30	1 year	16
R.P.	HEP 8.0 g	1953	3/6	2 years	1/6	0.04 g ID	>32[b]	10	1 year	8
L.J.	HEP 10.0 g	1953	16	9 months	1/6	2.0 g IM	>256[b]	20	1 year	16

[a] Positive in long incubation test.

systematic effort to explore ways of maintaining immunity indefinitely are tabulated in Table IX. In general, there seems to be some correlation between response to the first booster and that to subsequent boosters; 2 of the 4 persons with low-level response (1 : 4 or less) to the second booster also had responded poorly to the first, and one poor responder to the second also responded poorly to a third booster. Conversely, those with high-level response to the first ordinarily manifested a high-level response to the second booster. In 11 of the 18 instances the first booster stimulated immunity sufficiently so that readily demonstrable antibody was still present when the second booster was given from 5 months to 4 years later. However, at the time of the first booster (see Table VI) only 14 of 62 persons primarily immunized with HEP Flury vaccine 5 months to 4 years previously still had readily demonstrable antibody. To analyse further the crucial problem of antibody persistence after primary and booster immunization with HEP

TWO OR MORE BOOSTER INOCULA OF HEP FLURY VACCINE

booster			Third booster					Fourth booster				
inoculum (HEP Flury)	maximum response		interval	anti-body level	inoculum (HEP Flury)	maximum response		interval	anti-body level	inoculum (HEP Flury)	maximum response	
	level	day				level	day				level	day
0.4 g ID	4/6	10										
0.14 g ID	>32[b]	10										
0.08 g ID	>64	15?	1 year	>32	none							
0.04 g ID	>4[b]	10?										
0.04 g ID	64	20										
2.0 g IM	32?	10	2 years	128	0.08 g ID	>512	30					
0.33 g IM	>4[b]	10?										
0.03 g ID	4	10	1 year	0	0.14 g ID	2/6	30					
0.08 g ID	6	10	1 year	1/6	none							
0.33 g IM	>4[b]	10?										
0.33 g IM	>4[b]	10?										
2.0 g IM	4/6	30	1 year	0	0.08 g ID	8	15	1 year	0	0.08 g ID	8	30
2.0 g IM	4/6	10										
0.08 g ID	>512	15	1 year	>16	none							
0.33 g IM	>16[b]	10										
0.08 g ID	>32[b]	20	1 year	12	none							
0.002 g ID	>64	15	1 year	>4[b]	none							
0.33 g IM	>32[b]	20	1 year	6	0.08 g ID	96	15					

[b] Serum not available or not satisfactory for retesting to determine true neutralizing end-point.

Flury vaccine Tables X and XI were prepared. The numbers of observations are too small to permit very broad generalization, but it would appear that peak response to either a primary course or a booster inoculum of at least 1 : 32 titre is essential for persistence of readily demonstrable antibody for any significant period.

Restimulation of immunity induced by previous Pasteur treatment

Because of lack of knowledge and an understandable reluctance to tamper with established practice in dealing with a disease as serious as rabies, persons who have had a course of Pasteur treatment are often given a full course again on the occasion of a later exposure. Among the 136 persons listed in Tables XII and XIV, most of whom are veterinarians, 44 have had 2 or more courses and several have had 4 to 6 courses, sometimes

TABLE X. CORRELATION BETWEEN MAXIMUM ANTIBODY TITRE AFTER PRIMARY IMMUNIZATION WITH HEP FLURY VACCINE AND TITRES OBSERVED AT VARIOUS INTERVALS AFTER IMMUNIZATION [a]

Maximum titre (1:) after primary course	Antibody levels at indicated intervals after immunization															
	5 months				12 months				24 months				34 or more months			
	total	number with titre of 1:			total	number with titre of 1:			total	number with titre of 1:			total	number with titre of 1:		
		<4	4-7	8+		<4	4-7	8+		<4	4-7	8+		<4	4-7	8+
32+	2	1	1		2		1	1	2		1	1	2		1	1
8-31	3	2	1		4	1	1	2	1	1						
4-7	7	7			2	1		1	2	2			1	1		
Positive <4 [b]	8	7		1	16	15		1	6	6						

[a] This table includes a few observations on persons bled but not given a booster inoculum some time after the primary course and who therefore do not appear in Table VII.

[b] Undiluted serum protected some or all mice but 1 : 4 dilution did not.

at almost yearly intervals. Because persons undergoing repeated treatment are running an increased risk of demyelinating disease, Sellers[16] and a few others have advocated the use of an abbreviated course (perhaps 5 instead of 14 inoculations) in such instances. On reflection, it is evident that Pasteur treatment is a form of active immunization and that the general principles of immunology, including the anamnestic or recall phenomenon, should hold in this case as they do in the case of other forms of immunization.

TABLE XI. CORRELATION BETWEEN MAXIMUM ANTIBODY TITRE AFTER BOOSTER INOCULA OF HEP FLURY VACCINE AND TITRES OBSERVED AT SUBSEQUENT INTERVALS [a]

Maximum titre (1:) after booster	Antibody levels at indicated intervals after boosters															
	1 year						2 years						3 years or more			
	total	number with titre of 1:					total	number with titre of 1:					total	number with titre of 1:		
		0	<4	4-7	8-31	32+		0	<4	4-7	8-31	32+		0	<4	4-7	8+
128+	6			4	2		2				1	1	2			1	1
32-127	11	2		5	3	1	4	1		2		1					
8-31	1	1															
4-7	4	3	1				2	1		1							
Positive <4	1		1				1		1								

[a] Data entirely from Table IX

TABLE XII. ANTIBODY RESPONSE OBSERVED IN PERSONS WHO HAD PREVIOUSLY RECEIVED PASTEUR TREATMENT AND WHO WERE GIVEN A SINGLE BOOSTER INOCULUM OF HEP FLURY VACCINE

Individual	Prior Pasteur treatment		HEP Flury booster	Neutralizing antibody response[a] to booster on day				Evaluation[b]
	dates	years since last treatment		0	15	30	60	
R.B.[c]	No history	?	2.0 g IM	64	>256	>256	—	R
L.S.[c]	No history	?	2.0 g IM	0		>32	>32	R
E.H.G.	Unknown	?	0.16 g ID[e]	24 0 NI	—	>1024	512	R
D.Z.	Unknown	?	2.0 g IM	1/6 0 NI	0	1/6 0 NI	—	N
E.M.	1916	40	0.16 g ID	2/6	>32	32	128	R
J.C.	1917 or '18	38 or 39	0.08 g ID	0	0	3/6 0 NI	1/6	N
T.F.S.	1921	35	0.08 g ID[f]	0	—	0	—	N
P.T.[c]	1928	25	2.0 g ID	0	—	1/6 (P)	2/6	R
R.P.[c]	1927 or '28	25 or 26	2.0 g ID	1/6	0	0 (I)	—	N
M.A.[c]	1928	24	2.0 g ID	1/6	1/6	1/6 (N)	0	N
G.G.W.	1932	24	0.08 g ID	2/6	>512	>512	380	R
Ds.[d]	1934	21	2×0.08 g ID	0	7.2[g]	—	2	R
V.D.M.	1935	21	0.08 g ID	16	>4100	>4100	>4100	R
T.R.C.	1935	21	0.16 g ID[e]	0	—	48	—	R
A.R.	1936	20	0.16 g ID[e]	0	—	0 (P)	—	R
R.H.D.	1934	20	2.0 g IM	256	>1024	512	—	R
R.O.M.	1937	19	0.08 g ID	0	96	56	52	R
M.S.	1937	19	0.08 g ID[f]	128	—	256	200	R?
T.L.[c]	1934	18	2.0 g IM	8	>128	>128	>128	R
T.J.J.	1933, '36, '38	18	0.16 g ID[e]	24	—	256	128	R
A.W.[c]	1936	17	2.0 g IM	0	6/6	>128	—	R
C.Mc.[c]	1935	17	2.0 g IM	0	2/6	0 (P)	—	R
H.A.W.	1937, '39	17	0.16 g ID[e]	0	200	32	—	R
W.J.O.	1940	16	0.08 g ID	0	256	64	16	R

[a] NI = Neutralization index, long incubation test; (P) = positive in long incubation test; (N) = Negative in long incubation test; (I) = inconclusive in long incubation test.
[b] Evaluation: R = response; R? = uncertain response; N = no response.
[c] Prisoner-volunteer
[d] Muguga, Kenya
[e] Centrifuged vaccine
[f] Young-embryo vaccine
[g] 10th-day bleeding
[h] 76th-day bleeding
[i] Serum not available or not satisfactory for retesting to determine true neutralizing end-point.

TABLE XII. ANTIBODY RESPONSE OBSERVED IN PERSONS WHO HAD PREVIOUSLY RECEIVED PASTEUR TREATMENT AND WHO WERE GIVEN A SINGLE BOOSTER INOCULUM OF HEP FLURY VACCINE (continued)

Individual	Prior Pasteur treatment		HEP Flury booster	Neutralizing antibody response[a] to booster on dav				Evaluation[b]
	dates	years since last treatment		0	15	30	60	
D.H.T.	1940	16	0.08 g ID	256	1500	1024	512	R
T.W.B.	4 courses (last in 1940)	16	0.16 g ID[e]	0	64	32	32	R
E.J.S.	1937, '40	16	0.08 g ID[f]	24	—	180	—	R
C.v.H.	1940	16	0.08 g ID	<16	—	—	128	R
E.L.M.	1941	15	0.08 g ID	<16	—	40	24	R
G.K.C.	1941	15	0.16 g ID[e]	0	—	—	48	R
K.V.J.	1918, '42	14	0.08 g ID	6	>512	512	>512	R
R.A.H.	1941, '42	14	0.08 g ID	8	—	>64	—	R
C.F.R.	1942	14	0.08 g ID	0	—	32	—	R
A.L.B.	1941, '43	13	0.16 g ID[e]	48	>512	—	—	R
E.S.	1943	13	0.16 g ID[e]	2/6	—	64	—	R
J.R.C.	1943	13	0.08 g ID	32	—	2048	—	R
L.C.	1943	13	0.08 g ID[f]	16	—	128	—	R
E.B.	1943	13	0.08 g ID[f]	0	—	96	—	R
S.G.F.	1944	12	0.16 g ID[e]	0	—	96	—	R
W.L.S.	1938, '40, '44	12	0.16 g ID[e]	6	380	—	128	R
S.R.B.	1944	12	0.08 g ID	8	—	—	>64	R
Can.[d]	1946	11	2×0.08 g ID	0	66[g]	27	—	R
A.M.S.	1945	11	0.08 g ID	32	760	870	440	R
C.H.J.	1941, '45	11	0.08 g ID	0	—	128	—	R
J.C.F.	1945	11	0.16 g ID[e]	0	—	—	16	R
R.J.[c]	1942	10	2.0 g IM	0	96	>256	—	R
F.B.W.	1946	10	0.08 g ID	32	190	—	—	R
C.F.	1947	9	0.08 g ID	256	1000	1200	3600	R
C.A.P.	1944, '45, '46, '47	9	0.08 g ID	32	64	128	64	R

[a] NI = Neutralization index, long incubation test; (P) = positive in long incubation test; (N) = Negative in long incubation test; (I) = inconclusive in long incubation test.
[b] Evaluation: R = response; R? = uncertain response; N = no response.
[c] Prisoner-volunteer
[d] Muguga, Kenya
[e] Centrifuged vaccine
[f] Young-embryo vaccine
[g] 10th-day bleeding
[h] 76th-day bleeding
[i] Serum not available or not satisfactory for retesting to determine true neutralizing end-point.

TABLE XII. ANTIBODY RESPONSE OBSERVED IN PERSONS WHO HAD PREVIOUSLY RECEIVED PASTEUR TREATMENT AND WHO WERE GIVEN A SINGLE BOOSTER INOCULUM OF HEP FLURY VACCINE (continued)

Individual	Prior Pasteur treatment		HEP Flury booster	Neutralizing antibody response[a] to booster on day				Evaluation[b]
	dates	years since last treatment		0	15	30	60	
J.P.H.	1947	9	0.08 g ID	6	1180	1260	1350	R
H.C.H.	1947	9	0.16 g ID[e]	0	>512	256	—	R
J.D.D.	1945, '47	9	0.08 g ID	11	—	20	—	R?
R.L.J.	1947, '48	8	0.08 g ID	56	—	75	—	R?
W.T.K.	1940, '48	8	0.08 g ID	160	>512	>512	>512	R
R.B.L.	1948	8	0.08 g ID	2048	4096	>4096	>16 400	R
C.E.B.	4 courses (last in 1948)	8	0.16 g ID[e]	64[e]	—	—	256	R
J.H.Y.	5 courses (last in 1948)	8	0.16 g ID[e]	0	1,6	—	4/6	R
Bu.[d]	1932, '48	7	2×0.08 g ID	<20	224[g]	563	144[h]	R
L.H.P.	1941, '49	7	2×0.08 g ID	190	>1024	>1024	—	R
W.L.T.	1948, '49	7	2×0.08 g ID	512	—	>1024	>1024	R
P.J.L.	1949	7	0.16 g ID[e]	0	760	512	—	R
R.R.	1947	7	2.0 g IM	48	>128	80	—	R
C.R.D.	1947, '49	7	0.16 g ID[e]	128	8000	4096	–	R
W.K.[c]	1946	6	2.0 g IM	0	0	0	—	N
T.K.[c]	1949	6	0.16 g ID[e]	12	>64	96	—	R
J.M.L.	1930, '35, '40, '47, '50	6	0.08 g ID	1024	>8200	>8200	3000	R
J.B.V.	1930, '48, '50	6	0.08 g ID	340	>2048	>2048	2048	R
B.G.D.	1950	6	2×0.08 g ID	0	>128	>128	>128	R
J.S.D.	1942, '46, '50	6	0.16 g ID[e]	128	>1024	512	512	R
H.L.G.	1950	6	0.16 g ID[e]	0	>2048	1024	380	R
R.O.S.	1950	6	0.08 g ID	0	—	96	—	R
P.J.E.	1943, '47, '52	5	0.08 g ID	128	3100	1700	—	R
R.W.R.		5	0.08 g ID	21	—	230	—	R
D.L.T.	1951	5	0.08 g ID	0	—	64	—	R
C.J.B.	1947, '52	4	0.16 g ID[e]	380	>2048	—	—	R

[a] NI = Neutralization index, long incubation test; (P) = positive in long incubation test; (N) = Negative in long incubation test; (I) = inconclusive in long incubation test.
[b] Evaluation: R = response; R? = uncertain response; N = no response.
[c] Prisoner-volunteer
[d] Muguga, Kenya
[e] Centrifuged vaccine
[f] Young-embryo vaccine
[g] 10th-day bleeding
[h] 76th-day bleeding
[i] Serum not available or not satisfactory for retesting to determine true neutralizing end-point.

TABLE XII. ANTIBODY RESPONSE OBSERVED IN PERSONS WHO HAD PREVIOUSLY RECEIVED PASTEUR TREATMENT AND WHO WERE GIVEN A SINGLE BOOSTER INOCULUM OF HEP FLURY VACCINE (concluded)

Individual	Prior Pasteur treatment		HEP Flury booster	Neutralizing antibody response[a] to booster on day				Evaluation[b]
	dates	years since last treatment		0	15	30	60	
Man.[d]	1951	4	2×0.08 g ID	0	>100[g]	>50	—	R
G.C.T.	1946, '52	4	0.08 g ID	9	—	45	—	R
Kae.[d]	1952	3	2×0.08 g ID	<6	>100[g]	112	—	R
Wik.[d]	1952	3	2×0.08 g ID	>191	>50[g]	>50	—	R?
Asm.[d]	1950, '51, '52	3	2×0.08 g ID	957	2350[g]	3640	—	R
Uns.[d]	1952	3	2×0.08 g ID	55	853	957	—	R
J.M.D.	1953	3	0.08 g ID	0	1700	1200	1200	R
G.M.A.	1953	3	0.08 g ID	40	—	>1024	3600	R
M.G.O.	1950	3	2.0 g IM	0	>64[i]	>64[i]	>64[i]	R
J.B.M.	1953	3	0.16 g ID[e]	11	—	69	—	R
C.H.P.	6 courses (last in 1953)	3	0.16 g ID[e]	56	—	282	—	R
W.D.S.	1953	3	0.16 g ID[e]	14	—	125	—	R
A.E.W.	1947, '49, '51, '53	3	0.16 g ID[e]	45	—	166	—	R
C.B.[c]	1952	2	2.0 g IM	8	147	104	—	R
S.A.P.	1953, '54	2	0.08 g ID	380	2048	1500	1800	R
Men.[d]	1953	2	2×0.08 g ID	3	>50[g]	>50	—	R
Sch.[d]	1954	1	2×0.08 g ID	3	>100[g]	>50	—	R
Tre.[d]	1954	1	2×0.08 g ID	18	1124[g]	138	—	R
Thi.[d]	1954	1	2×0.08 g ID	28	>50[g]	>50	—	R
L.E.I.	1947, '55	1	0.08 g ID	128	3000	4100	760	R
G.W.K.	Jan. 1955, May 1955	1	0.08 g ID	512	>4096	1440	2048	R
E.L.M.	1939, '54, '55	1	0.16 g ID[e]	20	—	38	—	R?
And.[d]	1952, '53, '54, '55	<1	2×0.08 g ID	112	145[g]	112	—	R?
McL.[d]	1955	<1	2×0.08 g ID	0	196[g]	112	—	R
N.O.H.	1956	<1	0.16 g ID[e]	9	—	18	—	R?
B.C.Y.	1956	<1	0.16 g ID[e]	16	—	>128	—	R
K.K.D.	1956	<1	0.08 g ID		—	0	—	N
E.D.K.	1956	<1	0.08 g ID	166	—	282	—	R?

[a] NI = Neutralization index, long incubation test; (P) = positive in long incubation test; (N) = Negative in long incubation test; (I) = inconclusive in long incubation test.
[b] Evaluation: R = response; R? = uncertain response; N = no response.
[c] Prisoner-volunteer
[d] Muguga, Kenya
[e] Centrifuged vaccine
[f] Young-embryo vaccine
[g] 10th-day bleeding
[h] 76th-day bleeding
[i] Serum not available or not satisfactory for retesting to determine true neutralizing end-point.

With this in mind, a strenuous effort was made to find persons who had received Pasteur treatment and to persuade them to receive a single inoculum of HEP Flury vaccine — as a booster, it was hoped. It was felt that, because of its freedom from central nervous system tissue, it should be safe with regard to reactions of the central nervous system and, hence, if effective in recalling antirabies immunity, it might afford a completely safe method of treating re-exposed persons. A total of 136 persons were studied, whose histories of last treatment, known in 131 cases, ranged from a few months to 40 years. For the 103 persons whose sera were tested by the conventional method (using a constant, approximately 100 LD_{50} of virus), the responses following a single inoculum of from 0.08 g to 2.0 g of HEP

TABLE XIII. SUMMARY OF NEUTRALIZING ANTIBODY RESPONSES TO HEP FLURY BOOSTER INOCULA GIVEN TO PERSONS WITH HISTORY OF PREVIOUS PASTEUR TREATMENT

HEP Flury booster inoculum	Years since last treatment	Number of persons	Persons responding [a]		Range of maximum response titre (1:)
			number	%	
2.0 g IM	Unknown	3	2	67	>32 [c] - >256
	24-26	3	1	33	2/6 - >P-LT [b]
	17-20	4	4	100	2/6 - >1024
	6-10	3	2	67	>128 - >256
	2-3	2	2	100	>64 [c] - 147
	Total	15	11	73	2/6 - >1024
0.08-0.16 g ID	Unknown	1	1	—	>1024
	35-40	3	1	33	128
	20-24	5	5	100	0 (P-LT) [b] - >4100
	15-19	11	10 (11)	91 (100)	2/6 (P-LT) [b] - 1500
	10-14	16	16	100	32 - 2048
	5-9	25	23 (25)	92 (100)	4/6 - 16 400
	3-4	13	12 (13)	93 (100)	45 - 3640
	1-2	8	7 (8)	87 (100)	38 - >4096
	>1	6	2 (5)	33 (83)	18 - 282
	Total	88	77 (85)	87 (96)	0 (P-LT) [b] - 16 400
	All	103	88 (96)	85 (93)	0 (P-LT) [b] - 16 400

[a] Figures in parentheses include persons whose response was uncertain (R? in Table XII).
[b] P-LT = Positive in the long incubation test.
[c] Serum not available or not satisfactory for retesting to determine true neutralizing end-point.

Flury vaccine are tabulated in detail in Table XII and in summary form in Table XIII. In all, only 7 clearly failed to respond and 5 of these had received their last treatment 25 or more years previously. Further, 3 of the failures, including one " boosted " after 6 years, were in prisoner-volunteers whose histories of prior treatment could not be confirmed. In 8 instances, all with some residual antibody at the time of booster, the maximum post-booster antibody levels were not conclusively increased (i.e., twofold or greater). For the most part, the response titres were equal to, or considerably exceeded, those observed (see Table II) in persons undergoing an acceptable form of primary Pasteur treatment. Furthermore, as is better seen in Table XIII, small intradermal inocula were just as effective as the 2.0-g intramuscular inocula. The data for 33 persons, all given identical single intradermal inocula of 0.08 g, are shown in Table XIV. The sera of these individuals were tested only by screening 2 dilutions of the serum-virus mixture of the long incubation test, and the results are not subject to quantitative expression. Further, in 14 instances so much antibody persisted from the previous treatment that nearly all test mice were protected and no difference could be shown between the antibody levels before and 30 days after the HEP Flury booster inoculation. None the less, it is of interest that probable failure of response was observed in only 4 persons, of whom 3 had been treated 20 or more years previously. In general, therefore, it would seem that a single inoculum of HEP Flury vaccine can be relied upon, in the case of persons with histories of Pasteur treatment within a period of 20 to 25 years, to stimulate antibody titres at least equivalent to those normally observed after a full primary course of conventional Pasteur vaccine.

The observations just described provide, in the results of the titrations of the pre-booster sera, some additional and apparently unique data as to the persistence of antirabies neutralizing antibodies following Pasteur treatment. Before considering these data, it should be noted that 43 persons, or 32% of those with histories of treatment, admitted to having received 2 or more courses. While analysis of the response data showed no differences in response related to the number of courses of treatment, an important difference was noted in persistence of antibody. This is brought out in Table XV, which presents, for those treated but once and those treated two or more times, the titres of the pre-booster sera distributed according to the interval elapsed since the last course of treatment. After only one course, more than half retained antibody through 15 years and nearly three-fourths for 5 years. However, where 2 or more courses had been administered, all possessed antibody for 5 years and 90% retained it through 15 years. While it would obviously be desirable to have additional data and to be able to relate the data to the type and potency of the vaccine, it is evident that sero-immunity commonly persists for many years after Pasteur treatment, especially if more than one course has been given.

TABLE XIV. ANTIBODY RESPONSES OBSERVED IN CALIFORNIA VETERINARIANS WHO HAD PREVIOUSLY RECEIVED PASTEUR TREATMENT AND WHO WERE GIVEN A SINGLE INTRADERMAL BOOSTER INOCULUM OF HEP FLURY VACCINE (0.08 g)

Individual	Date of Pasteur treatment	Interval since last treatment (years)	Mortality ratios of mice [a] given indicated dilutions of serum-virus mixtures				Evaluation [b]
			pre-inoculation		30 days post-inoculation		
			undiluted	1 : 100	undiluted	1 : 100	
R.H.	Unknown	?	5/5	5/5	0/5	0/5	R
C.O.	1916	40	5/5	2/5	5/5	3/5	N
C.C.	1930	26	5/5	2/5	2/5	0/5	R
W.B.	1928, '33	23	5/5	5/5	5/5	3/5	R?
J.E.	1936	20	5/5	4/5	5/5	4/5	N
J.P.	1924, '29, '36	20	5/5	0/5	0/5	0/5	R
D.C.	1938	18	5/5	5/5	0/5	0/5	R
R.B.	1939	17	1/5	0/5	0/5	0/5	R?
A.I.	1940	16	0/4	0/3	0/4	0/5	R?
D.Mc.	1940	16	1/6	0/6	1/6	0/6	R?
W.R.	1937, '40	16	4/5	0/5	0/5	0/5	R
W.H.	1941	15	5/5	5/5	1/5	0/5	R
R.Mc.	1934, '41	15	0/5	0/5	0/5	0/5	R?
F.Mc.	1944	12	5/5	1/5	3/5	0/5	R
G.P.	1937, '44	12	NS [c]	NS [c]	0/5	0/4	R?
F.L.	1940, '46	10	6/6	0/6	0/5	0/6	R
W.K.	1947	9	5/5	0/5	1/5	0/4	R
E.M.	1942, '46, '47	9	1/6	0/6	0/6	0/5	R?
C.I.	1948	8	0/5	0/5	0/5	0/4	R?
R.H.	1948	8	0/5	0/6	0/5	0/6	R?
L.M.	1945, '48	8	0/6	0/6	0/6	0/6	R?
K.W.	1949	7	3/6	0/6	1/6	0/6	R
J.M.	1950	6	3/5	0/5	0/5	0/5	R
S.C.	1954	2	3/5	0/5	0/5	0/5	R
J.J.	1927, ?, '54	2	0/5	0/5	2/5	0/5	R?
J.P.	1955	1	0/5	0/6	0/6	0/6	R?
A.L.	1955	1	6/6	6/6	0/5	0/5	R
C.B.	1955	1	5/5	5/5	5/5	3/5	R?
T.C.	1955	1	4/6	0/6	0/4	0/6	R
W.D.	1955	1	0/5	0/6	0/6	0/6	R?
C.F.	1955	1	1/5	0/5	0/5	0/5	R?
E.P.	1938, '42, '55	1	0/5	0/6	0/6	0/6	R?
W.S.	1952, '53, '54, '55	1	4/5	0/5	0/5	0/5	R

[a] Mortality ratio is number of mice dying between 2 and 14 days after inoculation over number inoculated and alive 2 days later; method employed was the long incubation test.

[b] Evaluation: R = response; R? = uncertain response; N = no response.

[c] NS = no specimen.

TABLE XV. SUMMARY OF OBSERVATIONS ON PERSISTENCE OF NEUTRALIZING ANTIBODY AFTER PASTEUR TREATMENT

Number of treatment courses	Antibody level (1:)	Number of persons observed at indicated years since last treatment									
		<1	1-2	3-4	5-6	7-8	9-11	12-15	16-20	21-26	35-40
One	32+	1		3		2	3	1	3		
	8-31	2	3	2	2			2	1	1	
	4-7						1				
	<4 or P-LT [a]		7	1	1	3	1	3	4	1	1
	0	2	2	3	5	1	4	5	7	6	3
	total positive	3	10	6	3	5	5	6	8	2	1
	total	5	12	9	8	6	9	11	15	8	4
Two or more	32+	1	6	4	4	6	1	1			
	8-31		1	1			1	1	2		
	4-7							2			
	<4 or P-LT [a]					2	2	1	2		
	0					1	1		2	1	
	total positive	1	7	5	4	8	4	5	4	0	0
	total	1	7	5	4	9	5	5	6	1	0
One	percentage positive	60	76		57		55		43		25
Two or more		—	100		92		90		57		—

[a] P-LT = positive by long incubation test; <4 here means positive but with titre less than 1 : 4.

Discussion

The decision to attempt human immunization with HEP Flury chick-embryo-adapted virus was based on several considerations. As a living but highly attenuated virus strain, it might safely induce immunity in man as does the lower-passage Flury virus in dogs with a single inoculation. Also as a living viral agent, its antigenic character would not be modified or denatured as the result of either chemical or physical processes of inactivation. Third, as a vaccine prepared from chick-embryos from which the heads are removed, it is nearly completely free from central nervous system tissue and so should not provoke sensitization to the organ-specific antigen of the central nervous system which is believed to be responsible for treatment reactions. And finally, the extraneous material in the vaccine being of

embryonic origin, it should be of low antigenicity and relatively free from other undesirable allergic manifestations. The work to date on primary immunization with HEP Flury vaccine (567 courses) and recall immunization (223 booster inocula) provides reasonably solid support for the belief that the vaccine is intrinsically safe, and that the last two considerations mentioned are valid.

Unfortunately, the living state of the virus seems to be of little practical importance since the evidence suggests that it does not multiply extraneurally in man. This means that its antigenic effect depends entirely upon the original viral antigen present in the vaccine. Titres of HEP Flury virus obtained in embryos inoculated after 7 days' incubation range from 3.5 to 5.0 logs, with the result that the best lots of vaccine are of marginal potency. Hence, only the preparations with highest titres should be used as vaccines. The poor results associated with the inadvertent use of one low-titre lot (see Table I) underscores this point. So far the most practicable method of increasing virus titres in the embryo material has been the infection of embryos at a much earlier age. Peculiarly, the two lots of vaccine so prepared proved nearly completely devoid of antigenic power. This point obviously requires further study.

Because of the marginal potency of the vaccine, strenuous efforts were made to find ways to utilize the available antigen most efficiently. The available data led to the conclusion that the intradermal route was superior to the intramuscular and that, for a course to be completed within a reasonable period after known exposure (i.e., a maximum of 15 days), spacing of 4 inocula at 5-day intervals was optimum. A by-product of this study has been the demonstration that excellent results are achieved with a 4-dose course of subcutaneously inoculated Semple vaccine similarly spaced. However, on the basis of uniformity and level of antibody response as measured by the standard neutralization method, we are forced to admit that the best course of HEP Flury vaccine does not equal a course of good vaccine of conventional type (Harris or Semple). The duck-embryo vaccine[13] containing virus inactivated with β-propriolactone has the same theoretical advantages as to safety as does the HEP Flury vaccine; however, judging by the response to a 14-dose course (not tested with HEP Flury vaccine) it also is of marginal potency.

The question of the significance of neutralizing antibody in protection against rabies has been discussed elsewhere [1] and is far from settled. The possibility exists, on the basis of canine evidence,[17, 18] that the absence of readily demonstrable antibody does not necessarily signify lack of protection. In the case of poliomyelitis, Bodian [3] has shown that the smallest detectable levels of passively acquired antibody were sufficient to prevent central nervous system invasion by virus from the blood-stream. In this light, then, the level of antibody response may not be of critical importance although, admittedly, high titres of neutralizing antibody are comforting.

The additional observations on the development of active immunity in the presence of passive antibody support the previously indicated view that hyperimmune serum may exert a slight suppressive effect on active response. However, the difficulty of demonstrating this effect with vaccines of marginal potency suggests that, with fully potent vaccines, the phenomenon may not be of practical importance.

The greatest interest perhaps lies in the observations on restimulation of immunity, since the principles demonstrated are presumably applicable to all truly antigenic vaccines. The obvious potential application has to do with inducing and maintaining immunity indefinitely on a " before-exposure " rather than " after-exposure " basis in high-risk population groups. In previous reports[4, 5] considerable emphasis has been placed on the fact that a primary course of 2 or more doses of HEP Flury vaccine will reliably condition the recipient to respond to a single booster inoculum 5 or more months later, even when it fails to elicit a readily demonstrable immediate antibody response. While this remains essentially true, analysis of the responses of 64 persons given first boosters, and of the 18 given second or further boosters as well, indicates a fairly definite relation between the degree of response to primary immunization and that to the booster dose. Interestingly, Salk [14] recently reported a similar but even more definite phenomenon in relation to poliomyelitis vaccine.

Based on the results obtained in the present study, it is the opinion of the authors that the primary series of prophylactic immunizations against rabies of persons who have not undergone previous Pasteur treatment should consist of 4 intradermal injections of HEP Flury vaccine (0.08 g per dose) given at intervals of not less than 5 days. Subjects who have had Pasteur treatment should receive one intradermal injection for prophylactic purposes. Although data relative to the duration of immunity are scanty, it seems advisable at present to recommend revaccination with one dose of Flury vaccine every 2 years for persons falling in either of the above two groups and for whom long-continued maintenance of immunity is desirable because of their high risk of exposure. This may ensure the peak response titres of least 1 : 32 which seem to be necessary to maintain the presence of antibodies in the intervals between inoculations. Obviously, a continued and more systematic study of this problem of long-term immunization must be made.

Several aspects of the study of recipients of Pasteur treatment are of interest. First, perhaps, is the fact that antibody induced by such treatment commonly persists for as long as 5 years after a single course and for 15 or more years in persons who have received more than one course. The data here presented may be supplemented to a certain extent by those of Le Bell et al.,[11] who showed that the average titre for 69 persons (individual data not presented) was still quite high nearly a year after treatment. Of special interest is the superior persistence of antibody after a second or later

course of treatment. Since it presumably results from an enhanced response to re-treatment, it may be indicative of the pattern which will develop in persons given single booster inocula at intervals to maintain long-term immunity. It also clearly indicates that Pasteur treatment would provide a sound foundation upon which to build a programme of long-sustained immunity. Finally, treatment appears to condition the recipient so that at any time within 20 to 25 years he will respond rapidly to a single antigenic booster inoculum with antibody titres which often surpass those induced by a full primary course. The observation that in a single case such conditioning persisted for 40 years suggests that the failures observed among persons treated more than 25 years previously may have resulted from original lack of conditioning due to a non-potent vaccine rather than to " wearing-out " of the conditioned state.

RÉSUMÉ

Les auteurs ont exploré les possibilités qu'offre le vaccin Flury HEP (nombre élevé de passages sur œuf) pour l'immunisation primaire de sujets mordus ou fortement exposés aux risques de morsures par des animaux enragés. Le but poursuivi est triple: susciter une immunité rapide — nécessaire dans les cas où la période d'incubation est courte et la méthode Pasteur, de ce fait, inadéquate; éviter le danger de réactions démyélinisantes dues à l'inoculation de tissu nerveux hétérologue, en utilisant des cultures du virus sur œuf; créer une immunité active pour soutenir l'effet du sérum à forte teneur d'anticorps (hyperimmun) ou le remplacer lorsque son emploi est contre-indiqué.

Les études entreprises en 1954 ont montré que le virus Flury HEP, bien que vivant, ne se multiplie pas dans l'organisme humain. L'antigénicité du vaccin dépend directement de la quantité de virus contenue dans l'inoculat initial. Il faut en injecter des doses considérables pour obtenir des réponses assez constantes. On a cherché à améliorer son efficacité en l'administrant par voie intradermique plutôt que par voie intramusculaire. Une série de 4 injections à 5 jours d'intervalle paraît être la posologie la plus favorable. On doit reconnaître cependant que le schéma de vaccination le plus efficace par le vaccin Flury ne remplace pas un traitement complet par le vaccin classique de Harris ou Semple.

Le rôle des anticorps neutralisants dans la protection contre la rage est loin d'être élucidé. Il se peut, d'après les expériences faites sur les chiens, que l'absence d'anticorps décelables ne corresponde pas nécessairement à une absence de protection.

L'effet inhibiteur du sérum à forte teneur d'anticorps sur le développement de l'immunité active a été constaté. La question est à reprendre, en utilisant des vaccins très actifs.

Les observations les plus intéressantes peut-être, faites au cours de cette série d'études, ont trait à la stimulation de l'immunité par une ou plusieurs injections de rappel au moyen du vaccin Flury HEP. Des études antérieures avaient montré qu'une dose de rappel stimulait l'immunité, même dans le cas où une première inoculation n'avait pas donné lieu à une production d'anticorps décelable.

D'après les études récentes, les auteurs estiment que la première série d'inoculations prophylactiques chez des personnes n'ayant pas subi antérieurement le traitement selon la méthode Pasteur, devrait consister en 4 injections intradermiques de vaccin Flury HEP à intervalles de 5 jours. Une seule injection serait appliquée aux personnes ayant déjà été vaccinées par la technique de Pasteur. Une revaccination par une dose de vaccin Flury serait appliquée tous les deux ans aux personnes soumises à l'un ou l'autre des traitements précités.

Les anticorps suscités par la vaccination pasteurienne semblent durer au moins 5 ans après une série de traitement, et 15 ans au moins lorsqu'il y a eu plus d'un traitement. Toutes les expériences faites donnent l'impression que le traitement de Pasteur constitue une base solide, sur laquelle on peut construire une immunité de longue durée. A n'importe quel moment en l'espace de 20 ou 25 ans, une injection de rappel provoquera chez celui qui a subi un traitement pasteurien une teneur en anticorps supérieure à celle que suscite un traitement primaire complet.

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