JAUNDICE IN ARMY PERSONNEL IN THE WESTERN REGION OF THE UNITED STATES AND ITS RELATION TO VACCINATION AGAINST YELLOW FEVER

(PARTS II, III AND IV)[6]

By

W. A. SAWYER, K. F. MEYER, M. D. EATON, J. H. BAUER, PERSIS PUTNAM AND F. F. SCHWENTKER

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[6] This is the second of two installments.

II. THE NATURE OF YELLOW FEVER VACCINE AND ITS RELATIONSHIP TO INFECTIVE HEPATITIS

The conclusion was reached in part I of this report that the high prevalence of jaundice in certain troops in the western region of the United States in the spring and summer of 1942 was in some way related to previous vaccination against yellow fever. This conclusion was derived from observations during the preliminary field study of the investigative team and was clinched by the statistical analysis of numerous case records. It was not decided how the harmful lots of vaccine acted to produce jaundice. The remainder of this report will be occupied largely with this question. First there will be presented in part II a detailed account of the nature of the vaccine, the method of its manufacture, the source of the materials used, and the general experience in the United States and other countries with complications in the use of yellow fever vaccine and certain other serum-containing biologics. The attempts to find and identify an icterogenic agent in specimens of the suspect lots of vaccine or in materials from jaundiced patients will be left for presentation in part III. At the close of the preliminary field study, Dr. J. H. Bauer of the investigative team took over the principal responsibility for the compilations presented in part II.

A. The development of vaccination against yellow fever

Shortly after the transmission of yellow fever to laboratory animals by Stokes, Bauer and Hudson (1928) in 1927, there followed a series of accidental laboratory infections as recorded by Berry and Kitchen (1931). A number of these infections were fatal, including that of Dr. Stokes himself. Following these early deaths, intensified efforts were made in the laboratories in which yellow fever research was carried on to develop means of immunization, primarily for the immediate protection of those engaged in research under exceptional danger of infection and also as a prophylactic measure for combating the disease

at large. Among the first vaccines recommended were those of Hindle (1928, 1929) and of Aragão (1928), which consisted of an emulsion of ground infected monkey liver rendered noninfectious by various chemical inactivating agents such as formalin, phenol, chloroform, etc. It soon became evident, however, that none of these vaccines was effective as an immunizing agent, and it was shown subsequently by Findlay and MacKenzie (1936) and by Gordon and Hughes (1936) that inactivated virus was not capable of producing active immunity.

The first effective human vaccination against yellow fever was accomplished by Sawyer, Kitchen and Lloyd (1931, 1932) in 1931. The early vaccine consisted of active mouse-adapted neurotropic yellow fever virus and human convalescent serum. Since the introduction of this measure, there has not been a single known laboratory infection. However, this early method had a serious limitation in that very large amounts of immune serum would be needed if the measure was to be made available for the control of yellow fever in the field. Serious effort was made to substitute immune serum of animal origin for this purpose, but the results were not entirely satisfactory. Large numbers of very severe serum reactions occurred in those vaccinated. There were also instances in which the heterologous immune serum administered along with the virus apparently was excreted too rapidly, and frank, though mild, attacks of what was probably yellow fever followed, as reported by Soper and Smith (1938a).

From these observations it became obvious that large-scale vaccination against yellow fever as a prophylactic measure could not be undertaken until the virus itself was modified to such an extent that it could be safely used without immune serum. It is true that Laigret (1934) and other French workers used a mouse-passage neurotropic virus without immune serum for this purpose, but, as pointed out by Theiler and Whitman (1935) on the basis of experimental evidence, such a procedure seemed definitely dangerous. This was subsequently borne out by the field experience of Laigret himself (1936a, 1936b), as well as of Sorel (1936), Dezest (1937), Barraux, Montel and Bordes (1936), Mathis, Durieux and Mathis (1936), and Jadin and Arnaldi (1939).

Accordingly, a concentrated effort was made by the workers engaged in yellow fever research at the Laboratories of the International Health Division of The Rockefeller Foundation in New York to attenuate the virus to a greater extent than had been accomplished through serial passage in mice. Many different procedures were tried, but most of the work was done on the cultivation of the virus in vitro, especially in view of the success attained by Rivers and Ward (1931) with the virus of vaccinia by this method. At the beginning, great difficulty was encountered in attempts to cultivate yellow fever virus in tissue cultures, but Haagen and Theiler (1932) finally succeeded in growing a mouse-passage neurotropic strain in a medium consisting of normal monkey serum, Tyrode solution and chick-embryo tissues. Similar attempts with unmodified pantropic virus failed, until considerably later when Lloyd, Theiler, and Ricci (1936) eventually succeeded in cultivating the unmodified Asibi strain of yellow fever virus direct from the blood of an infected monkey in a medium consisting of mouse-embryo tissues, Tyrode solution and normal monkey serum. This was the original source of the so-called 17D virus at presen used for large-scale

human vaccination. It was the seventeenth experiment that proved successful, and the letters D and E were subsequently assigned by Theiler and Smith (1937a) to cultures containing chick and mouse embryos, respectively, for convenience in recording experimental data.

After the unmodified virus was established in tissue culture, constant effort was made to grow the virus in the presence of various types of tissues, all far removed from man, as it was hoped that in the presence of some of these tissues definite changes in the virulence of the virus might occur. After cultivation of the virus through 18 subcultures, the separate parallel series known as the D series was successfully established in a medium containing chick-embryo tissues in Tyrode solution and normal human serum. After the virus had grown in this medium through 50 subcultures, cultivation was continued with the modification that the head and the spinal cord were removed from the embryos before they were used in the cultures. This was done in the hope that prolonged cultivation in a medium containing only minimal amounts of nervous tissue might eventually reduce the inherent neurotropism of the virus.

The virulence of the virus in the different series of tissue cultures was tested in both monkeys and mice at frequent intervals. As shown by Lloyd, Theiler and Ricci (1936), the virus cultivated in the E series, i.e., grown in a medium containing mouse-embryo tissues, had already lost its viscerotropic virulence after 45 subcultures to such an extent that it no longer produced fatal infection in rhesus monkeys when injected in large doses intraperitoneally. Its neurotropic properties, on the other hand, had not changed appreciably, and when injected into monkeys intracerebrally, it persistently produced fatal encephalitis even after cultivation through 400 subcultures. Inasmuch as its inherent neurotropism had not, however, been enhanced as a result of in vitro cultivation, this 17E virus was used for human immunization in conjunction with immune serum by Lloyd, Theiler, and Ricci (loc. cit.), by Soper and Smith (1938a), and by Findlay and MacCallum (1937, 1938).

The virus grown in the D series of culture, i.e., in the chick-embryo medium, lost its viscerotropic virulence early. In fact, none of the cultures of this series tested in monkeys by either subcutaneous or intraperitoneal inoculation has ever produced fatal yellow fever infection in these animals. The neurotropic property of the virus, on the other hand, remained unchanged until after about 100 subcultures, when it was found to have suddenly diminished. The great majority of monkeys inoculated intracerebrally with the virus grown through a large series of subcultures, beginning with the one hundred and fourteenth, have survived, although occasional deaths have been observed even after the four hundredth subculture.

After exhaustive study in monkeys, Theiler and Smith (1937b) used the two hundred and twenty-seventh and two hundred and twenty-ninth subcultures of this virus without immune serum to vaccinate 8 human volunteers. The results were entirely satisfactory in that there were no serious reactions following the injection of the tissue-culture virus, and neutralizing antibodies in adequate concentration were found to be present in the blood of all of them 2 weeks after inoculation. Further preliminary trials on a limited number of human volunteers were conducted in Brazil by Smith, Penna, and Paoliello (1938), and by Soper and Smith (1938b), also with satisfactory results, before the 17D virus was finally adopted for large-scale vacci-

nation in Brazil. This virus was also sent for local production of yellow fever vaccine to the Wellcome Bureau of Scientific Research in London, to the Pasteur Institute in Paris, and to the Yellow Fever Laboratory maintained jointly by the Colombian Government and The Rockefeller Foundation in Bogotá, Colombia.

B. Complications arising from the application of the vaccine

As soon as vaccination had gained wider application, a number of serious complications were encountered. Besides some of those already mentioned above, 3 separate outbreaks of delayed jaundice and one of encephalitis were observed following vaccination against yellow fever. The essential data concerning these outbreaks are summarized below.

1. *England: postvaccination jaundice*

In 1937, Findlay and MacCallum (1937, 1938) reported that in the course of 4.5 years they had vaccinated approximately 2,200 persons against yellow fever and had observed 48 cases of jaundice occurring from 2 to 7 months after vaccination. In their 1938 report it was stated that there had been a total of 89 cases among 3,100 persons vaccinated, or just under 3 per cent, and in a later paper the number of cases was said to have totaled 96 (Findlay, 1940). In some groups the incidence apparently was as high as 15 per cent. Among physicians the incidence seems to have been especially high, as indicated by 8 cases among 33 medical officers vaccinated in Nigeria (Findlay and MacCallum, 1938). During this period the method of vaccination underwent several changes. The early vaccinations were done with a neurotropic virus derived from mouse brain together with immune serum. Then a tissue-culture virus of the 17E series was used also with immune serum; and later, the same virus was cultivated in the presence of chick-embryo tissue designated as "17EC," and used without immune serum. Jaundice was reported to have occurred following vaccination by all these methods, but the majority of cases were observed after the use of 17E virus, whether used with or without immune serum.

Findlay and MacCallum were firmly convinced that the jaundice was not caused by the yellow fever virus itself. In a later report Findlay, MacCallum and Murgatroyd (1939) again analyzed in detail the various factors involved in this episode and came to the conclusion that the causal agent of the jaundice was a virus, that it had been cultivated serially in tissue cultures in symbiosis with yellow fever virus, and that it must have been introduced into tissue cultures in association with human serum, presumably derived from a donor who was either in the incubation period or actually suffering from a mild subclinical attack of epidemic catarrhal jaundice. As a further proof of the virus nature of the icterogenic agent, they stated that it must have passed Seitz filters freely, as the yellow fever virus was filtered frequently during cultivation in vitro. As evidence that their yellow fever virus was contaminated with an icterogenic agent, they state that when they began to use the newly received 17D strain which was sent to them from the Laboratories of the International Health Division in New York in November, 1937, the jaundice ceased to appear and there were no further cases observed in the 8,000 persons vaccinated with this strain (Findlay, 1940).

2. *Brazil: postvaccination jaundice*

Soper and Smith (1938a) described the outbreak of jaundice which followed vaccination against yellow fever in 1936–1937 in Brazil. The virus used in this series was the 17E strain grown in tissue cultures containing mouse-embryo tissues in Tyrode solution-human serum mixture. This virus was used in conjunction with yellow fever immune serum either of animal or of human origin. Jaundice was limited to persons who had received hyperimmune monkey serum from two particular pools (no. 9 and 14), both prepared in Rio de Janeiro. Apparently over 30 per cent of those vaccinated with this material developed jaundice. No jaundice was observed in large numbers of persons vaccinated with the same virus but other immune serum pools. No definite conclusion was reached as to the cause of the jaundice, but in an unpublished report it was pointed out that at least one of these pools of hyperimmune monkey serum was prepared by the late Dr. Wray Lloyd at the time that he himself was suffering from a severe attack of jaundice. Early in 1937 this method of vaccination was discontinued and the field application of the 17D virus was introduced.

3. *Brazil: postvaccination jaundice, second outbreak*

During the period from February, 1937, to the middle of 1939 vaccination with the 17D virus was carried out on a large scale in Brazil. By June, 1939, 187 different lots of vaccine had been prepared and 1,300,000 persons vaccinated. The vaccine consisted of chick-embryo material infected with the 17D virus and suspended in normal human serum. In the preparation of most of these lots the serum was not inactivated. There was no jaundice observed following vaccination.

In October, 1939, a sharply localized outbreak of jaundice was observed. Investigation revealed that all the cases were limited to persons who had received a particular lot of vaccine, no. 467. The records showed that the normal human serum used in the preparation of this lot had been obtained from 9 blood donors, all healthy and without history of jaundice. The serum had been filtered twice through Seitz pads and inactivated by heating at 55 C for 30 minutes (J. A. Kerr, unpublished communication). The vaccine itself, however, was not filtered. About 27 per cent of those vaccinated with this lot developed jaundice in this particular locality 3 or 4 months after vaccination. Preliminary investigation of this outbreak revealed that the distribution of jaundice cases was peculiar in its localization. For example, the incidence of jaundice following the use of this lot was 32 per cent in Campos, Brazil; 22 per cent in Niteroy, Brazil; and 6 per cent in Bolivia. While the investigation of this particular outbreak was being carried on, certain changes in the technique of preparing the vaccine were made. Large-scale field vaccination was continued until May, 1940, when reports were received of another rise in the incidence of jaundice. By then 78 additional lots of vaccine had been employed, making 265 in all, and 600,000 more vaccinations had been done, making a total of 1,900,000 persons vaccinated since the introduction of 17D virus early in 1937.

A rapid field study of this new outbreak was made in May, 1940. It revealed that two more vaccine lots, no. 489 and 494, had resulted in delayed postvaccination jaundice. A detailed study of 1,072 cases in the outbreaks following the 3 lots with at least 32 deaths has recently been published by

Fox, Manso, Penna and Pará (1942). As in the case of lot no. 467, the distribution of jaundice among those vaccinated with the later two lots in different localities was very uneven. The case incidence in approximately equal numbers of persons vaccinated with lot no. 489 varied from 0 to 19 per cent, and with lot no. 494 from 0 to 10 per cent. The incidence for all 3 lots appeared to be highest in areas where a disease commonly designated as catarrhal jaundice was known to have been more or less endemic, and where the incidence of this disease among unvaccinated persons was higher than in other places. The experience of the Yellow Fever Viscerotomy Service revealed that the number of liver specimens from unvaccinated persons showing pathological lesions indistinguishable from those observed in postvaccinal fatal cases was greater in localities where the incidence of jaundice following vaccination was high than in places where jaundice was absent or rare. Another peculiarity noted was that both the incidence and the severity of the disease increased with the age of persons vaccinated. In addition, there appeared to be a tendency toward the grouping of cases in certain vaccinated households. This would be difficult to explain on the basis of random distribution unless there was a local contributory factor in these particular households.

On the basis of these peculiar epidemiological characteristics, the conclusion was reached that the disease was probably of double etiology, one of the etiological factors originating in the vaccine and the other contributed locally. The nature of both factors remained undetermined. It was thought that yellow fever virus itself played no part in it. This conclusion was based on the fact that jaundice had been observed in many persons known to have been immune to yellow fever before vaccination, as well as in some others who had failed to develop antibodies after vaccination. In addition, it was shown that an attack of jaundice did not affect the pre-existing yellow fever antibody level. As for the part played by the etiological factor contained in the vaccine, a conclusion similar to that of Findlay and his coworkers (1939) was reached. It was thought that the yellow fever virus used for the preparation of vaccine had become contaminated with an icterogenic agent during cultivation in tissue cultures, possibly before the inactivation of human serum was adopted. This agent was thought to have persisted in a symbiotic association with yellow fever virus through subsequent cultivation during which it only occasionally attained sufficient concentration to provoke a significant number of cases. This theory was based upon the fact that besides the 3 frankly icterogenic lots, there were 7 lots of vaccine which seemed to have been followed by a somewhat greater incidence of jaundice than all other lots. At any rate, it was concluded that in the absence of exact knowledge of the etiology of the disease produced, it would be difficult to guarantee that vaccines produced in the future would not be icterogenic.

A new strain of virus was sent from the New York laboratories, and the manufacture of vaccine was resumed at the end of 1940. With the change of the virus, the use of human serum in the preparation of the vaccine was discontinued. About 200,000 persons have been vaccinated with this new type of material in Brazil, and so far no jaundice has been reported.

4. *Brazil: postvaccination encephalitis*

In the summer of 1941 preliminary reports were received from Brazil indi-

cating that there was a considerable incidence of severe reactions following the application of the new type of vaccine from which human serum had been omitted. These reactions occurred approximately 2 weeks after inoculation and varied from a mild febrile illness to severe cases of encephalitis. There was mention of at least one death in a child in this series with frank manifestations of encephalitis. In fact, vaccination was suspended altogether for several months in 1941 while the outbreak was being investigated.

From the preliminary reports, no definite conclusion could be drawn as to the reason for this unusual occurrence except that it coincided with the change in the preparation of vaccine; that is, omission of human serum and the use of a new substrain of the 17D virus as seed. Fox, Lennette, Manso and Souza Aguiar (1942) have recently published a report in which they show this incident to have been due to the excessive neurotropism of the particular passaged substrain of 17D virus used.

5. *Colombia: vaccination without complications*

The 17D virus was sent to Colombia from the New York Laboratories of the International Health Division early in 1938. It has been used there for fairly large-scale vaccination ever since. The vaccine has been prepared from infected chick embryos and normal human serum. In 1938, 1939 and 1940 the serum was inactivated by heating to 56 C for 30 minutes; since 1941 the serum has been heated to 58 C for 1 hour. In addition to special precautions taken in the handling of the serum used for the vaccine, the donors who were volunteers in a Bogotá prison were observed for the possible appearance of jaundice for a month or more after bleeding. To date no undesirable reactions such as jaundice or encephalitis have been observed in Colombia although more than 600,000 persons have been vaccinated. This is considered significant, especially in view of the fact that hepatitis of unknown etiology is extremely common in Colombia; in fact, there are two areas where the disease is known to be endemic. One is the Cauca Valley near Concordia, and the other the Santa Marta region on the Atlantic Coast (Bauer and Kerr, 1933).

C. Extraordinary demands for vaccine created by war

Although the earlier types of vaccines, as well as the type used at present, were developed in the Laboratories of the International Health Division in New York, these laboratories had not been called upon to supply the vaccine on a large scale until June, 1940, when a request was received from the Subcommittee on Tropical Diseases of the Division of Medical Sciences of the National Research Council that 100,000 doses be maintained in stock for the armed forces of the United States. The Laboratories of the International Health Division were also requested to furnish the Army and Navy Medical Corps with a plan for production of this vaccine by the United States Government, with an estimate of costs and a list of competent personnel together with recommendations for the scientific control of the results of such vaccination. These requests were complied with, and it was understood that any vaccine supply would be given without charge as part of the contribution of The Rockefeller Foundation to the war effort. A communication was also received from the Surgeon General of the United States Public Health Service in which he expressed concern

TABLE 32

Monthly distribution of yellow fever vaccine in 1941 and first 4 months in 1942

Month	Doses				
	U. S. Army	U. S. Navy	Africa	Miscellaneous	Total
(1941)					
January	5,200		50,000	1,420	56,620
February	14,100		382,000	440	396,540
March	32,000		306,000	100	338,100
April	98,000	12,240	384,000	104,000	598,240
May	68,000		114,000	112,000	294,000
June	44,000	276,000		3,000	323,000
July	94,000	198,000		3,920	295,920
August	43,000	72,000			115,000
September	2,000				2,000
October	12,000	254,000		4,220	270,220
November	120,000		414,000	100,100	634,100
December	422,120	176,000	97,000	14,000	709,120
(1942)					
January	587,980	84,600		200	672,780
February	786,800	222,600		53,300	1,062,700
March	814,800	398,400	72,000	2,800	1,288,000
April	461,180	201,600			662,780
Totals	3,605,180	1,895,440	1,819,000	399,500	7,719,120
Vaccine returned	650,580	206,720			857,300
Net	2,954,600	1,688,720	1,819,000	399,500	6,861,820

regarding the supply of yellow fever vaccine available for civilians in the United States, pointed out the risks of the arrival of yellow fever in infectible territory of this country, and asked how much vaccine could be counted on as being available at all times in the Laboratories of the International Health Division. The figure of 200,000 doses was mentioned as being a desirable amount to hold in readiness for an emergency.

At about this time (May to December, 1940) an extensive epidemic of yellow fever occurred in the Nuba Mountains area of the Anglo-Egyptian Sudan (Kirk, 1941). Fifteen thousand cases were reported with more than 1,500 deaths. The proximity of this outbreak to the African war zones, together with the risks to the local civilian populations, resulted in requests from the British Government for very large amounts of yellow fever vaccine for use in Africa. To meet the requests from both the United States and the British Governments, the manufacture of the vaccine on a large scale was organized late in 1940 with Dr. Kenneth Goodner in charge. As shown in table 32, over 7 million doses were distributed between January, 1941, and April 10th, 1942, when the work was temporarily discon-

tinued because of the occurrence of jaundice in the United States Army. A change was made in the method of the manufacture, and during the remainder of 1942 over 4 million doses of the new type of vaccine were supplied, making 11 million in all up to the end of 1942.

D. Nature and source of the materials used in the preparation of the vaccine

When the Laboratories of the International Health Division were called on to prepare very large quantities of the vaccine, the scientists in charge were well aware of the complications described above, except for the most recently published, which had been encountered by other workers. All available data were carefully analyzed, and measures designed to avoid such occurrences were taken. The aim was to produce a vaccine that was entirely safe, efficient and of uniform quality.

1. *Unexpected changes in the behavior of yellow fever virus*

As indicated above, experience in England and Brazil suggested that the living modified yellow fever virus itself was the most vulnerable of all components going into the vaccine and that in the course of prolonged cultivation the strain was likely to become contaminated with an unknown agent which was prone to give rise to a delayed jaundice in vaccinated persons. In addition, experience had shown that unpredictable changes in the properties of the virus itself might at times occur when the virus was maintained in an active state through frequent passages in animals or often repeated tissue culture. These changes have been most puzzling, and factors responsible for them are not understood.

The first of such changes was noted by Bauer (unpublished observation) in Africa shortly after the yellow fever virus was first isolated from a human source. In those early days the present means of desiccation from the frozen state for preservation of the virus were not available, and the virus had to be maintained in mosquitoes between passages in monkeys. One morning it was noticed that during the night ants had entered a cage of infected mosquitoes and had killed all the insects. Dead mosquitoes, as well as some of the ants, were collected from the bottom of the cage. All were ground up together, and the suspension was injected into a monkey. Yellow fever virus was eventually recovered from the monkey, but in the process the virus had lost about 80 per cent of its initial virulence. After passage through 50 monkeys in series the original virulence had not been restored.

The second sudden change is that which occurred in the 17D virus during cultivation and which has been referred to above (Theiler and Smith, 1937a). To date it has not been possible to reproduce this change at will, either with the Asibi strain or with other strains of the virus, and the reasons for this fortunate change are still unexplained (Theiler, 1919).

A third change was observed by Soper, Smith and Penna (1939) in a substrain of the 17D virus which, for reasons still unknown, had lost much of its antigenicity during cultivation in tissue cultures between the three hundred and fifth and the three hundred and ninety-first subcultures. The loss of antigenicity was so great that 80 per cent of persons vaccinated with this virus failed to become immune. At the time it was thought that prolonged cultivation per se was responsible for the loss of antigenicity. That this is not necessarily

the case, however, was shown by Smith, Calderon-Cuervo and Leyva (1941) when they demonstrated that in another series of tissue cultures the antigenicity of the 17D virus remained unchanged after cultivation through the four hundred and fiftieth subculture.

A fourth change was reported by Penna and Moussatché (1939) in the highly virulent Asibi strain maintained in chick embryos by serial passages. The virus remained fully virulent until the nineteenth passage; at the twenty-ninth passage there was a marked diminution of viscerotropism, but the neurotropic properties appeared unchanged; at the thirty-ninth, forty-seventh and forty-ninth passage the neurotropism of the virus also had become greatly reduced.

A fifth change apparently occurred in another substrain of the 17D virus as reported by Fox, Lennette, Manso and Souza Aguiar (1942). This virus seems to have regained some of its original neurotropism and was responsible for the appearance of cases of encephalitis following vaccination.

2. *A seed virus system to assure uniformity of virus properties*

For the reasons stated above it was considered extremely important to have a virus with constant characteristics available for the preparation of vaccine. It was known that virus desiccated from the frozen state remains stationary and will not change on storage. Therefore, before vaccine manufacture on an extensive scale was undertaken in New York, a large supply of so-called seed virus was prepared. The virus-containing material used for this purpose was thoroughly mixed, distributed into ampules, desiccated, sealed and stored in a refrigerator. The substrain of the 17D virus used for this purpose was one which had been used in Colombia. This was chosen because it had produced no complications during the 4 years of use and in the immunization of 605,781 persons.

3. *The seed virus used*

The history of the particular substrain of 17D virus used in the Laboratories of the International Health Division for the preparation of yellow fever vaccine since the beginning of 1941 is shown graphically in figure 15. In detail the history is as follows: When the early experiments of Theiler and Smith (1937a) had indicated that the virus in the D series of tissue cultures had lost many of its neurotropic properties, a parallel series of cultures was initiated from the two hundred and fourth subculture of the original D series. In this new series, human serum was used with Tyrode solution in place of normal monkey serum, since this series was intended entirely for the preparation of vaccines for human use. This series is designated in the records as 17D204, and when reference is made to a particular subculture from this series used for the preparation of a given lot of vaccine, it has been the custom to add the number of passages in the new series to 204 to obtain the total number of subcultures that the virus has been carried through since the beginning of its cultivation in vitro.

Early in 1938, 340 tubes of yellow fever vaccine made in the New York laboratories were sent to the Yellow Fever Laboratory in Bogotá, Colombia. This lot was designated as NY75. It was made from the two hundred and twenty-first subculture of the 17D virus, substrain 17D204, which means that this particular virus had been cultivated through 204 subcultures in the original D series and 17 additional passages in

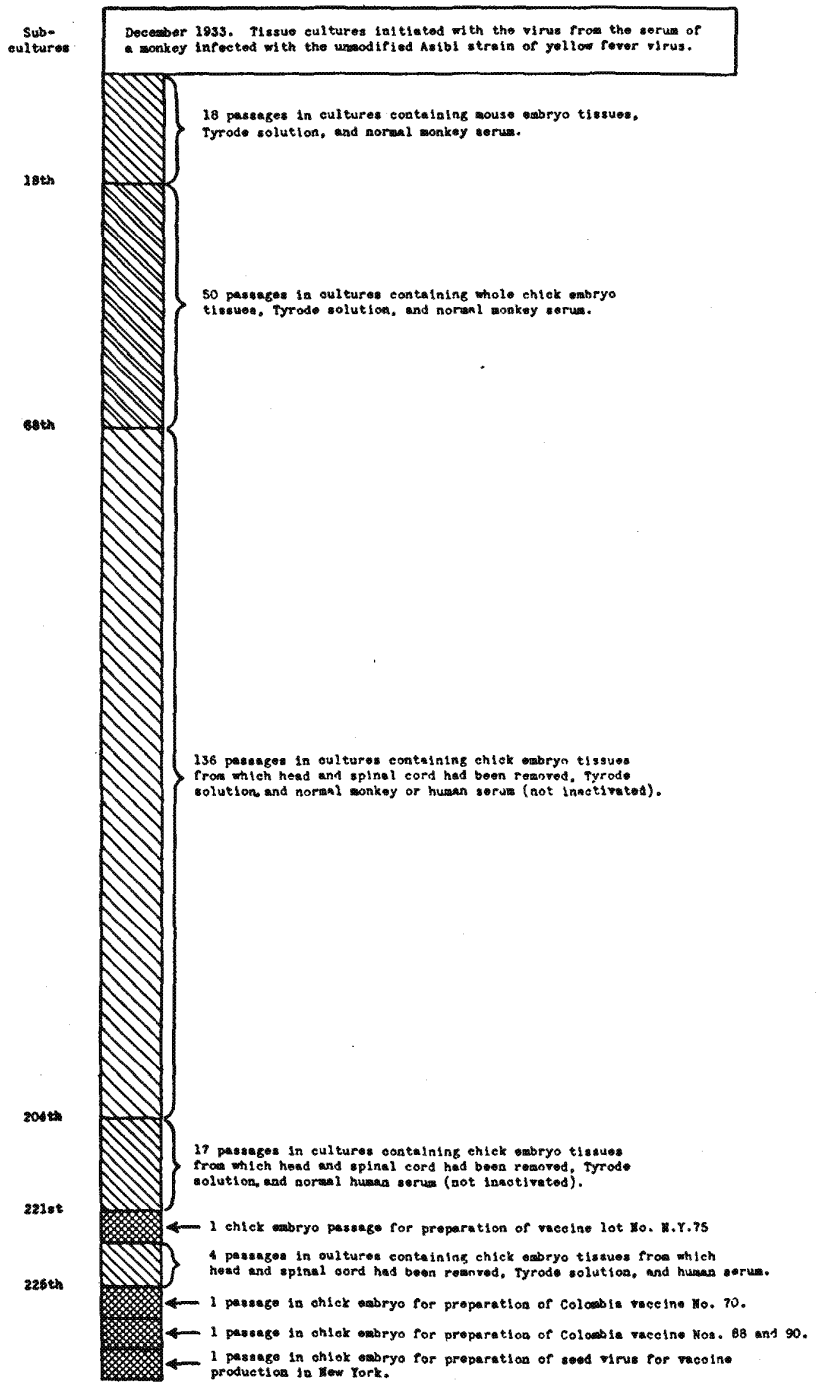

FIGURE 15. History of the seed virus used for the preparation of yellow fever vaccine.

the new 204 series. Liquid tissue culture of the two hundred and twenty-first passage was used to inoculate eggs for the preparation of vaccine lot NY75, which consisted of 20 per cent chick-embryo juice and 80 per cent undiluted normal human serum. The vaccine was passed through a Seitz filter and desiccated in the frozen state. The human serum, which was obtained from a professional blood donor in New York City, was not inactivated. Dr. J. C. Bugher, in charge of the Yellow Fever Laboratory, recently submitted details of the subsequent history in Colombia of the 17D virus in vaccine lot NY75.

According to Dr. Bugher's statement, the virus contained in this vaccine was passed through 4 additional subcultures in October, 1939. From the two hundred and twenty-fifth subculture, i.e., fourth in the Bogotá series, a lot of eggs was inoculated and made into vaccine of Colombia lot no. 70. In making this lot, according to Dr. Bugher's record, 13 per cent chick-embryo juice, 20 per cent distilled water, and 67 per cent normal human serum were used. The serum was inactivated for 30 minutes at 56 C and filtered; the vaccine was also filtered through a Seitz pad prior to desiccation. This lot of vaccine was used in Bogotá as seed virus for the preparation of subsequent vaccine lots used for large-scale human immunization in Colombia.

In the summer of 1940, two such vaccine lots, Colombia 88 and 90, were sent to the New York laboratories. In the preparation of these lots, Colombia vaccine no. 70 was used as seed virus for the inoculation of eggs. A 10 per cent chick-embryo suspension was made in normal human serum which had been inactivated by heating to 56 C for 30 minutes. The vaccine in these two lots was not filtered. In January, 1941, a very large lot of vaccine was made in our New York laboratories under serial lot no. 147–1. Colombia lot no. 88 was used as the seed virus for the inoculation of eggs used in the preparation of this lot. Lot no. 147–1 consisted of 20 per cent chick-embryo suspension in normal human serum which had been heated to 56 C for 1 hour. Vaccine of this lot has served as seed virus for the preparation of most of the vaccine lots prepared in the New York laboratories in 1941. In the spring of 1942, 5 new seed lots were made directly from Bogotá lot no. 88 from which human serum was omitted. It is expected that these new lots will be sufficient to prepare vaccine for several years.

4. *Source of eggs*

White Leghorn eggs have been used exclusively. They were received from two farms in New Jersey. These farms were thoroughly investigated, and assurance was received that the birds of these farms had been tested frequently for pullorum and fowl typhoid infection, and that the tests had been negative for a number of years. It was further claimed by the owners of these farms that the following diseases had been entirely absent: bacillary white diarrhea, fowl typhoid, fowl plague, fowl tuberculosis, fowl pox, fowl leukemia, fowl laringotracheitis. There had, however, been sporadic cases of fowl paralysis. The conditions on the poultry farms will be further discussed in part II, section J.

5. *The normal human serum*

Yellow fever virus cannot be initially grown in liquid tissue cultures without the addition of serum. During the 4 years of in vitro cultivation before final adoption for human immunization, the original 17D virus was grown in a medium of chick-embryo tissue in Tyrode

solution containing 10 per cent of normal monkey or human serum. Therefore, all branches of the 17D virus originated from a parent strain that had had a long association with human serum.

The reason for including human serum in the vaccine was to insure its efficacy as an immunizing agent. For the vaccine to be effective, the inoculum must contain a considerable amount of active yellow fever virus. The virus of yellow fever is one of the most labile viruses known. It was shown by Bauer and Mahaffy (1930) that the virus is rapidly inactivated when suspended in such common laboratory diluents as infusion broth, salt solution, Locke's solution, Ringer's solution, and distilled water, and that the addition of serum to these diluents delays the inactivation process very greatly. These findings have been subsequently confirmed by many workers engaged in yellow fever research. Without serum it seemed that yellow fever virus could not be cultivated in tissue culture, but it was subsequently found that after the virus has once been adapted to in vitro cultivation, serum might be replaced with chick-embryo juice.

The concentration of virus in liquid tissue cultures is generally very low regardless of the type of medium used. In infected chick embryos the concentration is considerably higher, although it is still very much lower than in the blood of an infected monkey, for example. A considerable amount of the virus is usually lost during the processing of the infected chick-embryo material into finished vaccine. Most of this loss generally occurs during desiccation.

It was known that vaccine containing only inactivated virus was entirely inert as an immunizing agent (Findlay and MacKenzie, 1936; Gordon and Hughes, 1936), and that when the concentration of active virus in the vaccine was low, a considerable proportion of vaccinated individuals failed to become immunized. This was clearly demonstrated by the observation of Fox, Kossobudzki and da Cunha (1943) on a relatively small number of persons who were given small and varying amounts of vaccine subcutaneously. Their results were as follows:

Mouse lethal doses of virus in inoculum	Persons developing immunity	Persons not immunized	Totals
11.5	18	0	18
2.9	9	10	19
0.7	1	18	19
0.18	2	18	20

Mice are not very susceptible to yellow fever, especially to the 17D strain, and it is presumed that in a single mouse lethal dose there must be a considerable amount of active virus. However, from the field experience on a much larger number of vaccinated persons, these workers concluded that an optimum amount of virus per human immunizing dose is about 500 mouse lethal doses, which would assure the immunization of nearly all persons inoculated. Although published only recently, these facts have been known for several years (Smith, Penna and Paoliello, 1938). Serum was added to the vaccine in an effort to preserve a maximum of virus until the vaccine was actually injected, and particularly to protect the vaccine during preparation and after it had been redissolved for use. Human serum was chosen because it gives less foreign protein reaction than serum of animal origin.

Normal human serum for tissue cultures, or for the preparation of vaccine on a limited scale, was usually obtained from professional blood donors secured through the Blood Transfusion Better-

ment Association in New York City. When the Laboratories of the International Health Division were called upon in 1941 to supply vaccine on a very much larger scale, it became necessary to arrange for additional sources of serum, as it was required at the rate of 8 to 10 liters per week. Dr. Thomas B. Turner, Professor of Bacteriology, Johns Hopkins School of Hygiene and Public Health, Baltimore, Md., very kindly agreed to secure human serum for us from volunteers in Baltimore. These donors consisted largely of medical students, internes, nurses and laboratory technicians, all presumably healthy. The serum was separated in Baltimore and brought to New York once a week.

E. Technique of the preparation of yellow fever vaccine

1. *Incubation and inoculation of eggs*

Until the latter part of 1941, eggs were incubated for 8 days on the farm prior to delivery. This expediency was resorted to because incubator space was too limited for the large number of embryos needed. It had a definite disadvantage in that the transportation of 8-day-old embryos resulted in a considerable mortality and retarded further development of others. To overcome this difficulty, special egg incubators of large capacity were obtained, and since then eggs have been incubated in the laboratories throughout the necessary period. Incubation is at 99.5 F with 75 to 80 per cent humidity. Position of the eggs is changed every 6 hours. Each shipment of eggs receives a serial number which follows the eggs and pulp until the material is processed into finished vaccine.

On the ninth day of incubation the eggs are candled, and those containing active living embryos are divided into sublots of 360. Each sublot is given a number for identification. Since September, 1942, the eggs containing satisfactory embryos have been immersed prior to inoculation for a period of from 5 to 15 minutes in a sterilizing mixture consisting of:

Tap water	17,000 ml
Sodium bicarbonate	100 grams
Clorox (concentrated sodium hypochlorite solution)	3,000 ml

The eggs are then removed with gloved hand from the sterilizing bath and set with the air sac uppermost on fiber packs of the type used in the poultry industry. For inoculation of each sublot of 360 eggs, one ampule containing 5 ml of desiccated seed virus is opened and the contents are rehydrated with 20 ml of physiological saline. A hole large enough to permit the passage of a 25-gauge needle is then pierced with a sharpened dental probe or pick in the shell of each egg above the air sac. With a combined thrusting and twisting motion the pick is brought sharply against the center of the upper shell surface. The pick is sterilized after every two piercings by dipping in alcohol and flaming. Inoculations are made with 0.5-ml tuberculin syringes fitted with 1.5-inch 25-gauge needles. The needle is inserted vertically to a depth of about 1 inch and 0.03 ml of the seed virus suspension is injected. After inoculation the openings in the eggs are sealed with phenolized sterile paraffin, and the position of the eggs is changed to the lateral. They are then incubated at 99 F for 4 days to permit the multiplication of virus. During this period their position is not changed.

2. *Harvesting of infected embryos*

Both the inoculation and the harvesting is done in special, air-conditioned, sterile rooms. The air circulating in

these rooms is filtered through oiled glass fiber filters and passed over banks of ultraviolet light. Before these operations the rooms and furniture are thoroughly washed with a strong soap and lysol mixture. All persons taking part in inoculation or harvesting wear sterilized masks, caps and gowns.

Before harvesting, the eggs are candled with great care. Besides the movements of the embryo itself, the state of its blood vessels as seen in candling is taken into consideration in choosing suitable embryos for vaccine. By the thirteenth day of incubation the blood vessels usually have extended to within 1.5 centimeters of the pointed end of the egg. The character of these vessels has proved a very reliable index of the state of the embryo. Eggs showing no such vascular extension, those in which this extension seems frayed, and those showing other abnormalities are discarded. This candling usually eliminates 40 to 60 eggs from a sublot of 360.

The eggs are then sponged off with 70 per cent alcohol containing 10 grams of potassium iodide and 3 grams of metallic iodine per 1,000 ml. In order to open the shell sterilely, a ring is burned around each egg at a point approximately 2 millimeters below the lower margin of the air sac. For this purpose, an oxyacetylene torch is used in connection with a driving mechanism which slowly rotates the egg. This apparatus was originally devised by Penna (1939) and later modified by Pickels (1942). The point of burning is chosen so as to allow the removal of the membrane along with the shell cap. The flame is sharply pointed and intense, and the egg revolves at a speed sufficient to prevent excessive heating of the egg content. The egg top is removed by a twisting thrust of a sterile scalpel along the line of burning. The scalpel is sterilized by flaming alcohol every 4 eggs unless a suspicious egg happens to be opened, in which case the scalpel is sterilized immediately. The embryo is removed from the shell with sterile blunt forceps and transferred to a covered shallow sterile pan. After the desired number of embryos is collected in a pan, each embryo is lifted by means of sterile curved forceps, the head is severed with sterile dentate scissors just back of the eyes, and the body proper allowed to fall into a sterile beaker. From a given sublot of eggs, 1,200 to 1,600 ml of embryos are usually obtained.

3. *Milling of embryos*

The next step in the procedure is the maceration of the embryonic tissue into a fine pulp. A colloidal mill is used for this purpose. The most suitable one found is a 2-inch type-L carborundum mill manufactured by the Premier Mill Corporation, Geneva, N. Y., which is designed primarily for the blending of face creams, etc. This mill consists essentially of a carborundum stator provided with an adjustable clearance from a carborundum rotor which turns at a very high speed. For sterilization purposes, the mill proper is removed from the frame and the head is set to give the widest possible clearance. The whole assembly is then wrapped in towels and autoclaved for 2.5 hours. After cooling, the mill is mounted and the driving belt placed in position. The stator is then adjusted to give a clearance of about 0.01 millimeter from the rotor, this position having been previously determined and marked on the adjusting screw. The mill is operated by a one-horsepower electric motor, with the speed of the rotor in operation approaching 16,000 r.p.m. As soon as the mill is brought to full operating speed, 1 liter of sterile distilled water is passed through for the

purpose of washing away any fluid that may have condensed from sterilization. A beaker of embryos without heads is then introduced into the funnel, and sterile distilled water is added at the rate of 25 ml for each 100 ml of embryo volume. The addition of water is necessary to facilitate free flow of the embryo pulp through the mill. The milled pulp is collected in 2-liter wide-mouthed Pyrex bottles. Between the millings of successive beakers of embryos within a given sublot, the mill is rinsed with 500 ml of sterile distilled water; and between successive sublots, each consisting usually of 3 beakers, 1 liter of sterile distilled water is used for rinsing. Frequent rinsing not only clears the mill of cartilage and other cellular debris, but also washes away any bits of tissue which may be contaminated with bacteria. Until subsequent results have proved to the contrary, at this stage it is assumed that each sublot may be contaminated. Therefore, the object of thorough rinsing between sublots is to avoid any carry-over into a sterile lot. In practice, this procedure has proved very effective.

4. *Sterility test and virus titration of pulp*

After milling, a sample is removed from each bottle for sterility tests and virus determination, and the bottle is then sealed airtight with a sterile rubber stopper. The pulp is shell-frozen and stored in a CO_2 cabinet at a temperature of approximately − 75 C. From the pulp sample, 1-ml, 0.25-ml, and 0.1-ml amounts are inoculated into fluid thioglycolate medium and incubated at 37 C. Cultures are observed on the second, fourth, and seventh days. Subcultures on agar plates are made from all tubes showing suspicious turbidity. Prior to July, 1942, beef infusion broth and agar were used in place of thioglycolate medium. For virus determination serial dilutions from each sample of pulp are inoculated intracerebrally into groups of 6 to 12 mice. After the mice have been observed for 21 days, the virus titer is interpreted in terms of the 50 per cent mortality endpoint of Reed and Muench (1938).

5. *Inactivation of human serum*

Serum was received from Baltimore once a week in bottles each containing approximately 500 ml. In New York a sterility test was done on the contents of each bottle. When the results of the test were found to be satisfactory, the serum was transferred by 100-ml pipettes into 250-ml Erlenmeyer flasks, 100 ml in each, for inactivation. The flasks were then immersed in a standard covered serological water bath to a depth of approximately 2 inches and held there by the weight of a lead ring around each. The temperature of the water in the bath was usually between 56 and 57 C, but dropped immediately after the flasks were immersed, unless the number of flasks was very small. The flasks were held in the water bath for 1 hour from the time when the temperature of the water regained its original level. Temperature of the serum during inactivation was not measured, but it is known that there was a certain time lag before the temperature of the serum in the flasks and that of the surrounding water reached an equilibrium. It is impossible now to evaluate this time lag in retrospect for the serum used in each particular lot of vaccine, as the length of this lag depended entirely on whether the serum at the time it was placed in the water bath was at icebox or room temperature. However, subsequent determinations indicated that this lag may have varied anywhere from 10 to 30 minutes, depending on the initial temperature of the serum and the total

number of flasks placed in the water bath in a single inactivation process.

After inactivation, a sterility test was again done on the serum from each flask before it was finally used in the vaccine.

6. *Processing of pulp into vaccine*

Frozen pulp which had proved sterile was thawed in another water bath at 37 C. The pulp was then transferred to 250-ml centrifuge bottles and centrifuged for 40 minutes at 2,000 r.p.m. The supernatant fluid was then passed through a column of sterile brass wire sieves, the finest being no. 60. This was done to remove fat and other insoluble material from the fluid. The clarified embryo juice was then transferred to large sterile bottles and diluted with inactivated human serum in the ratio of 4 parts of serum to 1 part of chick juice. This mixture constituted the concentrated vaccine.

In April, 1942, the following change was made in this procedure and is at present being continued. Human serum is omitted entirely, and the clarified embryo juice with its small content of water added during milling is diluted with an equal volume of sterile distilled water. The juice, of course, could be used as vaccine without additional dilution with water, but this practice was adopted merely as a matter of convenience in the distribution and desiccation of a desired amount of embryo juice in the ampules of the particular size which happened to be on hand in large quantities.

The vaccine was, and still is, prepared in ampules of two sizes. The ampule for 20 immunizing doses contains 1 ml of the concentrated vaccine, while the larger for 100 doses contains 5 ml of the same material. The term "vaccine lot" refers to material which is processed together as a unit and is of uniform composition. To each lot is assigned a serial number which is quite independent of the egg lot number or of the sublot designation. The liquid vaccine is poured into special Pyrex ampules in the conventional manner. It is then rapidly shell-frozen at the temperature of solid CO_2 and desiccated in the frozen state. The desiccation is accomplished by cold surface condensation using apparatus similar to that described by Bauer and Pickels (1940). Eight manifolds, each with 102 outlets, are used, thus allowing the desiccation of 816 ampules at one time. Eighteen to 20 hours are necessary to desiccate this number of ampules. After the desiccation is complete, the vacuum is replaced with dry nitrogen filtered through a long column of sterile cotton and a chemical drying agent, and the ampules are sealed off with an oxygen flame.

7. *Tests of the vaccine for sterility and effect on animals*

Because of the peculiar nature of the vaccine with its content of living virus, there appears to be no method available by which absolute bacterial sterility can be guaranteed. The usual sterility tests are those ordinarily applied to biological products which contain one or more bacteriostatic agents, or which have been filtered. No preservative can be used in yellow fever vaccine, and filtration is likewise impracticable because of excessive loss of virus. The only method which would give complete assurance against bacteria would be the cultivation of the entire bulk of the vaccine. The present method, therefore, gives assurance only that the material is not seriously contaminated.

Random samples are taken from each lot of vaccine, the number depending

upon the original number of ampules in that lot as follows:

Ampules in lot	*Samples tested*
Less than 100	3
101–150	4
151–200	5
201–250	6
251–300	7
301–350	8
351–400	9
401–450	10

Meat-infusion broth containing 0.1 per cent of dextrose in Smith fermentation tubes was used for all sterility tests in vaccine made in 1941 and early in 1942. As a rule, 6 fermentation tubes were inoculated with each sample, the size of the inoculum varying from 2 to 0.5 ml of the rehydrated vaccine. All cultures were incubated at 37 C and held under observation for 7 days. Since the beginning of July, 1942, fluid thioglycolate medium has been used for sterility tests. It is used in deep tubes, 15 ml in each, but otherwise the technique is the same as that with the meat infusion broth.

The following standards have been set up for the interpretation of the sterility tests. The general experience has been that vaccine lots made from carefully selected pulp give no bacterial growth. If cultures from two or more ampules show growth, the lot of vaccine is discarded. If some of the cultures made from a single ampule show growth, especially when such growth seems unrelated to the amount inoculated, this is tentatively considered as due to technical mischance, and the entire test on a new set of samples is repeated. If any of the cultures in the repeat series show growth, the entire lot is discarded; but if all are negative, the vaccine is released.

The titration of yellow fever virus in the finished vaccine is done in mice in the customary manner, as with the pulp earlier. In addition, rhesus monkeys are inoculated intracerebrally from each lot or a pool of 2 or 3 lots of finished vaccine, using 0.5 ml of the rehydrated vaccine for the inoculum. The monkeys are kept under observation for 30 days. They are bled daily from the second to the seventh day after inoculation, and groups of mice are inoculated intracerebrally with the blood. This is done for the purpose of determining the amount of virus circulating in the blood following inoculation. The monkeys are bled again about 2 weeks after inoculation, and their serum is tested for neutralizing antibodies. The great majority of monkeys inoculated intracerebrally with the vaccine show fever for 1 to 3 days, but as a rule they do not appear ill. Occasionally some die of yellow fever encephalitis. Where death of the test animal occurs, the vaccine lot under test is not released.

Other safety tests in animals include inoculation of mice intraperitoneally with 0.5 ml of rehydrated vaccine, and the inoculation of guinea pigs with 1-ml amounts by the same route. The mice are kept under observation for 2 weeks, and the guinea pigs for 30 days. When any test animal dies, every effort is made by autopsy and bacteriological studies to determine the cause of death. Where there is any doubt as to the safety of the vaccine the lot is discarded.

8. *Diluent for yellow fever vaccine*

With each ampule of vaccine, a bottle of sterile diluent for the rehydration and dilution of the vaccine is supplied. Formerly this diluent consisted of physiological salt solution, but since human serum has been omitted from the vaccine, this diluent has consisted of 0.2 per cent of sodium chloride in distilled water. It has been known for some time that yellow fever virus survives better in dis-

tilled water than in 0.9 per cent salt solution (Bauer and Mahaffy, 1930). On the other hand, when distilled water was used as a diluent, complaints were received that the injection was painful. Hence, a compromise on 0.2 per cent was made. The diluent is put up in bottles of two sizes. The smaller bottle contains 11 ml and is designed for the rehydration and dilution of the vaccine from the smaller ampules containing the dried equivalent of 1 ml of fluid vaccine. This amount of vaccine dissolved in 11 ml of diluent results in a dilution of somewhat more than 1 in 10; and 0.5 ml of this dilution is used as an immunizing dose. Twenty doses should be available per ampule and bottle, since the extra milliliter of diluent covers the loss occurring in manipulation. The larger bottle contains 55 ml of diluent and is supplied with the larger ampule of vaccine containing 5 ml of the concentrated material. It is used for rehydration and dilution in the same proportion as in the case of the smaller bottle. It yields 100 immunizing doses and leaves an additional 5 ml to cover any loss. The diluent is supplied in rubber-capped bottles of the Army Medical School design. It is sterilized by autoclaving, and sterility tests are done in the same manner and on the same proportion of bottles as with the ampules of the vaccine itself.

9. *Control of efficacy and safety*

While the vaccine was being supplied to the armed services of the United States, every effort was made to secure detailed information as to its behavior under field conditions. Each ampule was wrapped in a printed sheet giving instructions regarding rehydration and application of the vaccine, as well as requesting information on any untoward reactions following its administration. It also contained a request for a specimen of blood taken 1 month after vaccination from 1 per cent of persons vaccinated, for the determination of the presence of yellow fever antibodies by the mouse protection test as an indication of successful immunization.

In table 33 are shown data on 141 lots of vaccine made from December 20th, 1940, to March 18th, 1942, with indication of the destination of each lot. The total number of immunizing doses per lot and the seed virus used in the preparation of each lot are also given. It will be noticed that 3 different designations for seed virus are given. Of these, no. 147–1 has already been explained above; of the remaining two, C-88 is the Bogotá vaccine from which no. 147–1 was made, and no. 505 was made from no. 147–1. Six lots were made with C-88; 11 with no. 505; 5 with a mixture of no. 147–1 and 505; and 115 with no. 147–1.

No postvaccination serum specimens were received from Africa, but some tests are known to have been made at the Yellow Fever Institute at Entebbe, Uganda. Very few were sent in from the Navy. The Army submitted 1,405 specimens. Of these, only 72 failed to show neutralizing antibodies, indicating that about 95 per cent had been successfully immunized, as determined by a rather severe test.

Very few reports were received of unfavorable reactions following vaccination. The most common form of reaction consisted of general malaise, headache and slight fever, occurring on the fifth to eighth day after vaccination. This, however, was of short duration and seldom required hospitalization. Other forms of reaction were allergic in character, apparently due to the small amount of chick protein present in the vaccine. They varied in severity, but 3 instances apparently were so severe that it was necessary to administer

TABLE 33

Yellow fever vaccine supplied from the Laboratories of the International Health Division in New York during the period January 1st, 1941, to April 9th, 1942

Lot number	Seed virus	Date of preparation	Number of doses supplied to:				Total
			U. S. Army	U. S. Navy	Africa	Miscellaneous	
145–1	C–88	12–20–40			68,900		68,900
145–3	C–88	12–31–40	7,300			2,680	9,980
145–4	C–88	1– 2–41	4,000		5,000		9,000
145–5	C–88	1– 4–41			26,900	100	27,000
147–1	C–88	1– 9–41	220		147,500	3,180	150,900
203	505	1–23–41			48,000		48,000
204	C–88	1–28–41			24,000	2,700	26,700
206	505	1–28–41	8,000		58,300		66,300
207	147–1	2– 1–41			64,000		64,000
208	147–1	2– 6–41			47,800		47,800
209	147–1	2–13–41			97,800		97,800
210	147–1	2–14–41			16,000		16,000
211	147–1+505	2–19–41			140,000		140,000
212	147–1+505	2–20–41	7,780				7,780
213	147–1+505	2–26–41			162,500	600	163,100
214	147–1+505	2–27–41				96,700	96,700
215	147–1+505	3– 5–41			94,700	60,000	154,700
216	505	3–13–41			19,500	36,000	55,500
217	505	3–13–41	64,000		59,100		123,100
218	505	3–13–41			28,300		28,300
219	147–1	3–20–41	48,000		27,000		75,000
220	147–1	3–21–41	36,000		19,500		55,500
221	147–1	3–24–41	10,000		680	8,000	18,680
222	147–1	3–24–41	48,000		5,200		53,200
223	505	3–27–41			6,740	4,000	10,740
224	505	3–28–41	85,000		11,780		96,780
225	505	4– 1–41	140	96,780	16,940		113,860
226	505	4– 4–41	1,800	40,000	33,860	400	76,060
227	147–1	4– 8–41	5,000	61,660	4,000	4,400	75,060
228	147–1	4– 9–41	12,000	34,600	2,000	400	49,000
229	505	4–14–41	4,000	73,400		400	77,800
230	147–1	4–16–41		20,420			20,420
231	147–1	4–17–41	40	12,480		160	12,680
232	147–1	4–18–41	48,000	36,900		400	85,300
233	147–1	4–23–41	1,620	12,000		160	13,780
234	147–1	4–24–41	1,940	10,000		160	12,100
235	147–1	4–25–41	560	10,000		1,160	11,720
236	147–1	4–28–41	2,000	12,000		160	14,160
237	147–1	4–29–41	3,540	12,000		160	15,700
238	147–1	4–30–41		12,000			12,000

TABLE 33—*Continued*

Lot number	Seed virus	Date of preparation	Number of doses supplied to:				Total
			U. S. Army	U. S. Navy	Africa	Miscellaneous	
239	505	5– 1–41	1,780	40,000	2,700	1,960	46,440
240	147–1	5– 7–41	620	18,000	1,100		19,720
241	147–1	5– 8–41	20	11,560		160	11,740
242	147–1	5– 9–41	1,460	10,000		160	11,620
243	147–1	5–12–41		17,480		160	17,640
244	147–1	5–13–41	560	10,960		160	11,680
245	147–1	5–14–41	2,320	6,000	200	360	8,880
301	147–1	9– 2–41	3,160	12,000	1,600	220	16,980
303	147–1	9– 5–41	80	12,000	1,600		13,680
304	147–1	9– 8–41	8,860	6,000	2,400		17,260
305	147–1	9– 9–41	380	72,000	3,600		75,980
306	147–1	9–11–41	2,980	28,000	48,000		78,980
307	147–1	9–13–41	500		11,000	4,000	15,500
308	147–1	9–15–41	3,680	12,000			15,680
309	147–1	9–16–41			71,000		71,000
310	147–1	9–18–41	1,900	24,000	1,200	44,000	71,100
311	147–1	9–20–41	1,820	12,000	9,500		23,320
312	147–1	9–22–41	8,780		4,100	8,000	20,880
313	147–1	9–24–41	16,120		2,000		18,120
315	147–1	9–29–41		76,000			76,000
316	147–1	10– 1–41	2,860	2,000	16,000		20,860
317	147–1	10– 3–41	3,380		20,000		23,380
318	147–1	10– 7–41	26,600	4,000		48,100	78,700
319	147–1	10– 9–41	2,920	4,000	46,000		52,920
320	147–1	10–11–41	3,600		24,000		27,600
321	147–1	10–14–41	48,840		18,000		66,840
322	147–1	10–15–41	2,700		76,000		78,700
323	147–1	10–17–41	25,800	48,000	4,000		77,800
324	147–1	10–20–41	1,080		47,000		48,080
325	147–1	10–22–41	10,840	6,000	8,000		24,840
326	147–1	10–23–41	4,740	30,000	12,000		46,740
327	147–1	10–27–41	640	42,000	12,000		54,640
328	147–1	10–29–41	1,840	12,000	12,000		25,840
329	147–1	11– 1–41	7,020	,6000	12,000		25,020
330	147–1	11– 3–41	38,400	4,000	36,000		78,400
331	147–1	11– 5–41	66,300			12,000	78,300
332	147–1	11– 7–41	74,600	4,000			78,600
333	147–1	11–11–41	13,300	12,000			25,300
334	147–1	11–13–41	13,080	2,000	8,000	2,200	25,280
335	147–1	11–14–41	68,480				68,480

TABLE 33—*Continued*

Lot number	Seed virus	Date of preparation	Number of doses supplied to:				Total
			U. S. Army	U. S. Navy	Africa	Miscellaneous	
338	147–1	11–22–41	74,400				74,400
340	147–1	12– 4–41	62,200				62,200
341	147–1	12– 9–41	29,100	48,000			77,100
342	147–1	12–11–41	12,960	12,000			24,960
343	147–1	12–19–41	6,560	24,000			30,560
344	147–1	12–23–41	13,900				13,900
345	147–1	12–29–41	77,700				77,700
346	147–1	12–31–41	55,000	4,100			59,100
347	147–1	1– 3–42	49,240	4,800			54,040
348	147–1	1– 5–42	17,400	18,200			35,600
349	147–1	1– 7–42	2,860	14,000			16,860
350	147–1	1–11–42	72,160	3,900			76,060
351	147–1	1–13–42	89,000				89,000
352	147–1	1–15–42	29,520	600			30,120
353	147–1	1–16–42	89,400	4,800			94,200
354	147–1	1–19–42	520	76,800			77,320
355	147–1	1–21–42	2,420	52,800			55,220
356	147–1	1–21–42	400	43,200			43,600
357	147–1	1–22–42	16,280			400	16,680
358	147–1	1–24–42	51,600				51,600
359	147–1	1–26–42	50,300				50,300
360	147–1	1–26–42	33,200				33,200
361	147–1	1–28–42	82,300				82,300
362	147–1	1–29–42	59,300				59,300
363	147–1	1–31–42	16,100		1,600	52,800	70,500
364	147–1	2– 3–42	59,600	40	9,400		69,040
365	147–1	2– 3–42	10,000	360	12,000	100	22,460
366	147–1	2– 4–42	6,540	140	12,000		18,680
367	147–1	2– 5–42	82,260		4,100		86,360
368	147–1	2– 7–42	81,600				81,600
369	147–1	2– 9–42	72,000	460	1,400		73,860
370	147–1	2–10–42	72,000	4,780	6,400		83,180
371	147–1	2–11–42	92,400	3,940		500	96,840
372	147–1	2–14–42	57,600	3,180	21,800		82,580
373	147–1	2–16–42	57,600	1,100	3,300		62,000
374	147–1	2–17–42	72,000	8,400			80,400
375	147–1	2–18–42	14,400	65,100		1,820	81,320
376	147–1	2–19–42		82,000			82,000
377	147–1	2–21–42		68,920			68,920
378	147–1	2–23–42		80,880			80,880

TABLE 33—*Continued*

Lot number	Seed virus	Date of preparation	Number of doses supplied to:				Total
			U. S. Army	U. S. Navy	Africa	Miscellaneous	
379	147–1	2–24–42	26,000	820		480	27,300
380	147–1	2–25–42	76,800	2,600			79,400
381	147–1	2–26–42	86,400	2,000			88,400
382	147–1	2–28–42	82,800	3,680			86,480
383	147–1	3– 2–42	72,000	4,300			76,300
384	147–1	3– 3–42	38,400	2,400			40,800
385	147–1	3– 3–42	67,200	4,500			71,700
386	147–1	3– 4–42	67,200	1,900			69,100
387	147–1	3– 5–42	57,600	2,300			59,900
388	147–1	3– 5–42	9,600	43,200			52,800
389	147–1	3– 6–42	22,000	16,000			38,000
390	147–1	3– 9–42	52,800	1,800			54,600
391	147–1	3– 9–42	33,600	3,600			37,200
392	147–1	3–10–42	67,200	1,400			68,600
393	147–1	3–10–42	57,600	4,700			62,300
394	147–1	3–12–42	29,180	79,900			109,080
395	147–1	3–14–42		40,300			40,300
396	147–1	3–14–42	14,400	34,200			48,600
397	147–1	3–16–42	72,000	10,600			82,600
398	147–1	3–17–42	72,000	12,700			84,700
399	147–1	3–18–42	57,600	7,800			65,400
Total doses			3,605,180	1,895,440	1,819,000	399,500	7,719,120

adrenalin. No clear-cut cases of encephalitis were reported, and the vaccine seemed highly satisfactory until the appearance in March, 1942, of delayed jaundice in certain troops several months after vaccination. When this occurred about 7 million doses of vaccine had been distributed during the preceding 15 months.

F. Jaundice in the military forces in the United States and elsewhere following vaccination against yellow fever

1. *Postvaccination jaundice in the United States Army*

As stated in part I of this report, information was received in March, 1942, that in United States Army personnel there was a considerable amount of jaundice which appeared to be associated with certain lots of yellow fever vaccine used for immunization. In Circular Letter no. 95, War Department (1942), dated August 31st, 1942, the weekly incidence of jaundice in the army personnel stationed in Continental United States was given for the period from March 7th to August 15th, 1942. It was shown that the peak was reached during the week of June 20th, with a steep decline in the incidence thereafter.

We are indebted to Col. Bayne-Jones, and to Maj. Walker, for information regarding the number of cases reported on questionnaires in relation to the dif-

ferent lots of vaccine used for immunization of army personnel. These data, obtained from the questionnaires returned to the Office of the Surgeon General by medical officers in the field and from the Surgeon General's correspondence up to the end of 1942, are shown in table 34. There are 117 lots of vaccine and 26,771 cases of jaundice shown in this table. The number of doses of vaccine in each lot is also shown, and the computed case rate per 1,000 doses of vaccine is indicated. An inspection of this table reveals at once that a certain amount of jaundice appears to be associated with the majority of the lots of vaccine. This is brought out strikingly in figure 16 which is based on this table. While "normal" occurrence of jaundice in troops accounts for some of the spread of case incidence over many lot numbers of vaccine administered, numerically, the great majority of the cases appear to be related to only 7 lots. Hence, an attempt was made to divide the vaccine lots into different categories according to their icterogenic property.

Because several important factors are still unknown, it is impossible to determine from the information available at present how many of the vaccine lots possessed definite icterogenic property. It is not known how many persons were actually vaccinated with each lot of vaccine, nor is it known exactly how many cases followed the use of each lot. The tabulation in table 34 does not include all cases that occurred in the Army as a result of yellow fever vaccination, as many of the questionnaires, especially from units stationed overseas, have been slow in reaching the Office of the Surgeon General, and in some instances the lot number of the vaccine is not known. Furthermore, it is not known whether mild, unhospitalized cases of jaundice

TABLE 34

The relationship of jaundice in the Army to yellow fever vaccine

Vaccine lot number	Total number of doses supplied	Number of doses returned	Number of doses net issue	Number of cases reported	Case rate per 1,000 doses net issue
145-3	7,300		7,300	1	0.14
145-4	4,000		4,000		0.00
147-1	220		220		0.00
206	8,000	100	7,900		0.00
212	7,780		7,780		0.00
217	64,000		64,000	24	0.38
219	48,000		48,000	36	0.75
220	36,000		36,000	16	0.44
221	10,000		10,000	1	0.10
222	48,000		48,000	11	0.23
224	85,000	300	84,700	41	0.48
225	140		140		0.00
226	1,800		1,800	1	0.56
227	5,000		5,000		0.00
228	12,000		12,000	3	0.25
229	4,000		4,000	5	1.25
231	40		40		0.00
232	48,000	200	47,800	8	0.17
233	1,620		1,620	2	1.23
234	1,940		1,940		0.00
235	560		560		0.00
236	2,000		2,000	1	0.50
237	3,540		3,540	1	0.28
239	1,780		1,780		0.00
240	620		620		0.00
241	20		20		0.00
242	1,460		1,460		0.00
244	560		560		0.00
245	2,320		2,320		0.00
301	3,160	20	3,140	3	0.96
303	80		80		0.00
304	8,860		8,860	6	0.68
305	380		380		0.00
306	2,980		2,980	4	1.34
307	500	60	440	6	13.64
308	3,680	400	3,280	2	0.61
310	1,900	60	1,840	2	1.09
311	1,820		1,820	3	1.65
312	8,780	320	8,460	20	2.36
313	16,120	260	15,860	13	0.82
316	2,860		2,860	10	3.50
317	3,380	100	3,280	18	5.49
318	26,600	100	26,500	54	2.04
319	2,920	40	2,880	27	9.38
320	3,600	40	3,560	8	2.25
321	48,840	20	48,820	11	0.23
322	2,700		2,700	12	4.44
323	25,800		25,800	11	0.43
324	1,080	40	1,040	5	4.81
325	10,840	140	10,700	12	1.12

TABLE 34—*Continued*

Vaccine lot number	Total number of doses supplied	Number of doses returned	Number of doses net issue	Number of cases reported	Case rate per 1,000 doses net issue
326	4,740	160	4,580	3	0.66
327	640	40	600	1	1.67
328	1,840	160	1,680	1	0.60
329	7,020	1,540	5,480	93	16.97
330	38,400	200	38,200	78	2.04
331	66,300	2,000	64,300	1,750	27.22
332	74,600	200	74,400	75	1.01
333	13,300	60	13,240	5	0.38
334	13,080	540	12,540	428	34.13
335	68,480	200	68,280	3,861	56.55
338	74,400	3,500	70,900	2,235	31.52
340	62,200	4,400	57,800	277	4.79
341	29,100	100	29,000	56	1.93
342	12,960	1,440	11,520	1	0.09
343	6,560	240	6,320	2	0.32
344	13,900	6,320	7,580	11	1.45
345	77,700	6,200	71,500	112	1.57
346	55,000	300	54,700	110	2.01
347	49,240	3,140	46,100	19	0.41
348	17,400	7,880	9,520	1	0.11
349	2,860	560	2,300	13	5.65
350	72,160	8,940	63,220	302	4.78
351	89,000	7,200	81,800	212	2.59
352	29,520	2,000	27,520	3	0.11
353	89,400	7,600	81,800	25	0.31
354	520		520		0.00
355	2,420	200	2,220	12	5.41
356	400		400	2	5.00
357	16,280	2,220	14,060	27	1.92
358	51,600	9,200	42,400	28	0.66
359	50,300	4,700	45,600	20	0.44
360	33,200	100	33,100	78	2.36
361	82,300	8,400	73,900	61	0.83
362	59,300	2,600	56,700	79	1.39
363	16,100	200	15,900	72	4.53
364	59,600	13,640	45,960	111	2.42
365	10,000	3,700	6,300	18	2.86
366	6,540	1,200	5,340	10	1.87
367	82,260	5,620	76,640	4,643	60.58
368	81,600	2,300	79,300	5,360	67.59
369	72,000	16,800	55,200	5,288	95.80
370	72,000	21,900	50,100	325	6.49
371	92,400	19,420	72,980	34	0.47
372	57,600	6,300	51,300	149	2.90
373	57,600	10,500	47,100	75	1.59
374	72,000	23,000	49,000	31	0.63
375	14,400	3,000	11,400	6	0.53
379	26,000	8,920	17,080	12	0.70
380	76,800	5,300	71,500	45	0.63
381	86,400	14,700	71,700	48	0.67
382	82,800	19,360	63,440	21	0.33
383	72,000	33,000	39,000	9	0.23
384	38,400	9,500	28,900	8	0.28
385	67,200	12,100	55,100	20	0.36
386	67,200	27,400	39,800	23	0.58

TABLE 34—*Continued*

Vaccine lot number	Total number of doses supplied	Number of doses returned	Number of doses net issue	Number of cases reported	Case rate per 1,000 doses net issue
387	57,600	13,200	44,400	19	0.43
388	9,600	800	8,800	7	0.80
389	22,000	12,420	9,580	2	0.21
390	52,800	26,000	26,800	54	2.01
391	33,600	15,500	18,100	2	0.11
392	67,200	48,300	18,900	3	0.16
393	57,600	21,900	35,700	12	0.34
394	29,180	7,960	21,220	1	0.05
396	14,400	12,400	2,000	1	0.50
397	72,000	43,100	28,900	2	0.07
398	72,000	44,000	28,000	1	0.04
399	57,600	52,600	5,000		0.00
Totals	3,605,180	650,580	2,954,600	26,771	9.06

were recorded in the returned questionnaires. For these reasons it is impossible to compute the exact case rate per number of persons actually vaccinated with each lot, or to compare this rate with the normal jaundice incidence in the Army in order to decide which of the vaccine lots were icterogenic. However, it would seem reasonable to assume that the case rate per 1,000 doses of vaccine shown in table 34 would have to be multiplied by at least a factor of 2 which would allow for cases not included in this table and also for the unavoidable wastage which must have occurred in the administration of the vaccine. We are indebted to Col. Bayne-Jones for the information that the normal incidence of jaundice in the United States Army during the last 10 years has been between 1 and 2 per 1,000 per annum. If the case rates shown in table 34 are multiplied by 2 as suggested and compared with the normal incidence, then it will at once appear that a considerable number of the vaccine lots possessed icterogenic property to varying degrees. In fact, of the 177 lots of vaccine listed in this table, 47 show a case rate of more than 1 per 1,000 doses of vaccine

FIGURE 16. Postvaccination jaundice incidence in the United States Army.

Category of vaccine	Lots	Doses net	Percentage of total doses	Cases	Percentage of total cases
1. Highly icterogenic, with case rate between 10 and 100 per 1,000 doses of net issue	9	433,080	14.7	23,664	88.4
2. Moderately icterogenic, with case rate between 1 and 10 per 1,000 doses of net issue	38	910,620	30.8	2,441	9.1
3. Probably not icterogenic, with case rate less than 1 per 1,000 doses of net issue	70	1,610,900	54.5	666	2.5
Totals	117	2,954,600	100.0	26,771	100.0

supplied, which, when multiplied by 2, is more than 2 per 1,000, and therefore more than the normal jaundice rate in the Army.

On the basis of this analysis the vaccine supplied to the Army was divided into 3 categories according to the relative degree of icterogenesis as determined from the case rate per 1,000 doses issued as follows:

It will be seen from this tabulation that over 88 per cent of the cases of jaundice were associated with only 9 lots of vaccine, constituting 14.7 per cent of the total number of doses issued to the Army. These 9 highly icterogenic lots, set forth in table 35, were placed in category 1. According to the questionnaires, 23,664 cases of jaundice were associated with the 433,080 doses of vaccine in these 9 lots, giving an over-all incidence of 56.64 cases per 1,000 doses of vaccine issued. As seen from table 35, there is a considerable variation in the case rate of the different lots; from 13.64 per 1,000 doses for lot no. 307 to 95.80 per 1,000 doses for lot no. 369. It is true that the number of cases associated with lots no. 307 and 329 is small, but the amount of vaccine supplied from these lots was also small, and there seems to be no doubt that they belong in this category.

TABLE 35

Vaccine lots associated with jaundice incidence between 10 and 100 cases per 1,000 doses of net issue in the United States Army

Vaccine lot number	Doses net issue	Cases reported	Case rate per 1,000 doses
307	440	6	13.64
329	5,480	93	16.97
331	64,300	1,750	27.22
334	12,540	428	34.13
335	68,280	3,861	56.55
338	70,900	2,235	31.52
367	76,640	4,643	60.58
368	79,300	5,360	67.59
369	55,200	5,288	95.80
Totals	433,080	23,664	56.64

Category 2 includes vaccine lots which showed a case rate between 1 and 10 per 1,000 doses of vaccine issued. If these rates are multiplied by a factor of 2, as suggested above, it will be apparent that all have rates higher than the normal jaundice incidence in the Army. As shown in table 36, there are 38 lots in this category, consisting of a total of 910,620 doses, or 30.8 per cent of the total amount of vaccine supplied to the Army. There were 2,441 cases reported as associated with these lots, representing an over-all incidence of 2.68 cases per 1,000 doses of vaccine supplied; this number also represents 9.1 per cent of the total number of cases of jaundice. As seen from table 36, there is a considerable variation in the case rate among different lots of vaccine in this

category, and it may well be that some of the lots, especially those that have a very small number of cases associated with them, may not be icterogenic at all. On the other hand, many lots in this group have a relatively large number of cases associated with them and show a case rate several times higher than the normal jaundice incidence in the Army. Furthermore, it is known that the incubation period and other characteristics of the jaundice following the use of at least some of these moderately icterogenic lots were similar to those of the disease observed in connection with other, highly icterogenic lots of vaccine (Maj. Walker, personal communication). In the absence of a specific test by which an icterogenic lot of vaccine can be differentiated from a nonicterogenic lot, the entire group must be considered mildly or moderately icterogenic for the purposes of the present analysis.

Also for the purposes of the present analysis, the remaining 70 lots of vaccine, consisting of 1,610,900 doses, or 54.5 per cent of the entire amount supplied to the Army, are placed in category 3. There are 666 cases of jaundice reported as being associated with these lots, giving an over-all incidence rate of 0.41 per 1,000 doses. If this case rate is multiplied by a factor of 2, as already suggested for other categories, the incidence will then fall well into the case rate range normally occurring in the Army, and these lots must, therefore, be considered as nonicterogenic. In this category are also included 19 lots showing no cases at all. But, as shown in table 34, there was only a relatively small amount of vaccine supplied to the Army from these lots, and the total for these 19 lots is only 40,320 doses. It is doubtful that data on such a small amount of vaccine are of any particular significance.

It must be emphasized here that the

TABLE 36

Vaccine lots associated with jaundice incidence between 1 and 10 cases per 1,000 doses of net issue in the United States Army

Vaccine lot number	Doses net issue	Cases reported	Case rate per 1,000 doses
229	4,000	5	1.25
233	1,620	2	1.23
306	2,980	4	1.34
310	1,840	2	1.09
311	1,820	3	1.65
312	8,460	20	2.36
316	2,860	10	3.50
317	3,280	18	5.49
318	26,500	54	2.04
319	2,880	27	9.38
320	3,560	8	2.25
322	2,700	12	4.44
324	1,040	5	4.81
325	10,700	12	1.12
327	600	1	1.67
330	38,200	78	2.04
332	74,400	75	1.01
340	57,800	277	4.79
341	29,000	56	1.93
344	7,580	11	1.45
345	71,500	112	1.57
346	54,700	110	2.01
349	2,300	13	5.65
350	63,220	302	4.78
351	81,800	212	2.59
355	2,220	12	5.41
356	400	2	5.00
357	14,060	27	1.92
360	33,100	78	2.36
362	56,700	79	1.39
363	15,900	72	4.53
364	45,960	111	2.42
365	6,300	18	2.86
366	5,340	10	1.87
370	50,100	325	6.49
372	51,300	149	2.90
373	47,100	75	1.59
390	26,800	54	2.01
Totals	910,620	2,441	2.68

division of vaccine lots used by the Army into 3 categories according to the degree of their apparent icterogenesis is a purely arbitrary procedure based on incomplete information. It may well be that if the final figures eventually become available as to the number of persons actually vaccinated with each lot, and the number of cases of jaundice observed in these persons, some revision will have to be made. It is doubtful, however, that the picture will change very radically, and there seems to be no escape from the conclusion that a considerable number of the vaccine lots possessed a definite icterogenic property.

2. *Postvaccination jaundice in the United States Navy*

The discrepancy between the jaundice experience of the Army and that of the Navy, while both were deriving their yellow fever vaccine from the same source, was so striking that it must be explained before the evidence can be accepted that the vaccine was responsible for the epidemic in the Army. From table 33 it is evident that none of the definitely icterogenic lots was issued in quantity to the Navy, except lot no. 334, and that the amount of this lot was only 2,000 doses. A few hundred doses of lot no. 369 are shown on the record of issues to the Navy, but it is not known whether they were used. In any event, the total experience of the Navy with jaundice, viewed as a whole, seemed to be unremarkable and easily explained as due to ordinary infection with catarrhal jaundice. Analysis of the jaundice experience by lot numbers, however, confirmed the fact that the comparative freedom from jaundice was indeed due wholly to accidents of distribution and revealed a high percentage of jaundice in the small amount of vaccine used from the one lot (no. 334) incriminated by Army experience.

We are indebted to Captain T. J. Carter, M.C., of the Division of Preventive Medicine, Bureau of Medicine and Surgery, Navy Department, for information regarding the jaundice incidence following yellow fever vaccination in the United States Navy. According to this information 709 cases of jaundice were observed among 522,273 persons vaccinated, representing an over-all incidence of 1.4 cases per 1,000.

In table 37 and figure 17 is presented the distribution of 691 of these cases among 54 lots of vaccine. With one

TABLE 37

Postvaccination jaundice in the United States Navy

Lot number	Number doses issued	Number persons vaccinated	Number cases jaundice	Case rate per 1,000 vaccinated	Army rate per 1,000 doses issued
225	96,780	31,557	71	2.2	
226	40,000	20,302	34	1.7	
227	61,660	25,110	49	2.0	
228	34,600	15,635	30	1.9	
229	73,400	24,984	42	1.7	1.2
230	20,420	9,477	15	1.6	
231	12,480	3,057	2	0.7	
232	36,900	14,296	22	1.5	
233	12,000	4,518	9	2.0	1.2
234	10,000	4,332	4	0.9	
235	10,000	3,680	7	1.9	
236	12,000	1,107	1	0.9	
237	12,000	2,448	10	4.1	
238	12,000	6,714	2	0.3	
239	40,000	12,448	47	3.8	
240	18,000	6.194	9	1.5	
241	11,560	3,104	6	1.9	
242	10,000	4,607	9	2.0	
243	17,480	9,592	12	1.3	
244	10,960	8,432	31	3.7	
245	6,000	1,053	2	1.9	
301	12,000	3,926	3	0.8	
303	12,000	4,190	2	0.5	
304	6,000	1,263	1	0.8	
305	72,000	33,019	37	1.1	
306	28,000	8,602	0	0	1.4
308	12,000	2,499	0	0	
310	24,000	7,428	0	0	1.1
311	12,000	1,717	1	0.6	1.6
315	76,000	27,799	55	2.0	

TABLE 37—*Continued*

Lot number	Number doses issued	Number persons vaccinated	Number cases jaundice	Case rate per 1,000 vaccinated	Army rate per 1,000 doses issued
316	2,000	2,202(?)	8	3.6	3.5
323	48,000	5,242	1	0.2	
325	6,000	1,106	3	2.7	1.1
326	30,000	2,502	5	2.0	
327	42,000	10,790	4	0.4	
328	12,000	2,658	4	1.5	
329	6,000	7,090(?)	28	3.9	
332	4,000	6,873(?)	0	0	
333	12,000	1,837	10	5.4	
334	2,000	271	31	114.4	
341	48,000	5,172	2	0.4	1.9
342	12,000	4,529	1	0.2	
343	24,000	11,286	0	0	
348	18,200	1,223	1	0.8	
349	14,000	2,062	0	0	
350	3,900	2,384	1	0.4	4.8
354	76,800	16,023	4	0.2	
355	52,800	9,060	1	0.1	5.4
356	43,200	3,829	1	0.3	5.0
375	65,100	2,516	1	0.4	
376	82,000	1,168	0	0	
377	68,920	26,609	13	0.5	
378	80,880	12,825	1	0.1	
394	79,900	1,221	0	0	
Lot not stated		21,365	58	2.7	
Totals	1,645,740	464,933	691	1.4	

exception (lot no. 334), only lots used to vaccinate at least 1,000 persons are included in the table. The remaining 18 cases were scattered among other lots where the total number vaccinated was small, and they were therefore excluded from the present analysis. A study of this table reveals that, with the exception of lot no. 334 which was frankly icterogenic in the Army and also exhibited the same property in the Navy, the over-all incidence of jaundice following vaccination is low. As in the Army, here again the interpretation is somewhat handicapped by the fact that the entire Navy was vaccinated and the jaundice rate which would otherwise have prevailed is not known. However, in view of the fact that 41,139 persons, presumably distributed at random, were vaccinated, with 8 different vaccine lots without giving rise to a single case, the normal incidence of jaundice in the Navy cannot be very high, and it would seem improbable that this incidence exceeds 2 per 1,000. In setting up such an arbitrary yardstick, which we also believe is a conservative estimate for the analysis of postvaccination jaundice in the Navy, it becomes at once apparent that there were at least 7 lots, viz., no. 225, 237, 239, 244, 316, 325 and 333, that were associated with a jaundice rate higher than this hypothetical average and might possess a moderate degree of icterogenesis. If the cases following vaccination with lot no. 334 are excluded, it will be found that 30 per cent of all cases were associated with these 7 lots, even though the number of persons vaccinated represent only 15 per cent of the total.

There are certain discrepancies in this table which we are not in a position to explain. There are, for example, 3 lots, no. 316, 329 and 332, showing a larger number of persons vaccinated than there was vaccine in these lots issued to the Navy. This may have been due to an error in recording lot numbers. In table 37 are included 11 lots which also had been supplied to the Army, and the case rates with 7 of those, viz., no. 306, 310, 311, 341, 350, 355 and 356, are considerably higher in the Army, even though the rate in the Army was computed on the number of doses issued and not on the number of actual vaccinations.

3. *Postvaccination jaundice elsewhere*

Although the total amount of vaccine supplied to the United States Navy or sent to Africa and to foreign governments elsewhere was more than twice the amount received by the United States Army, no outbreaks of jaundice were reported. It appears to have been a

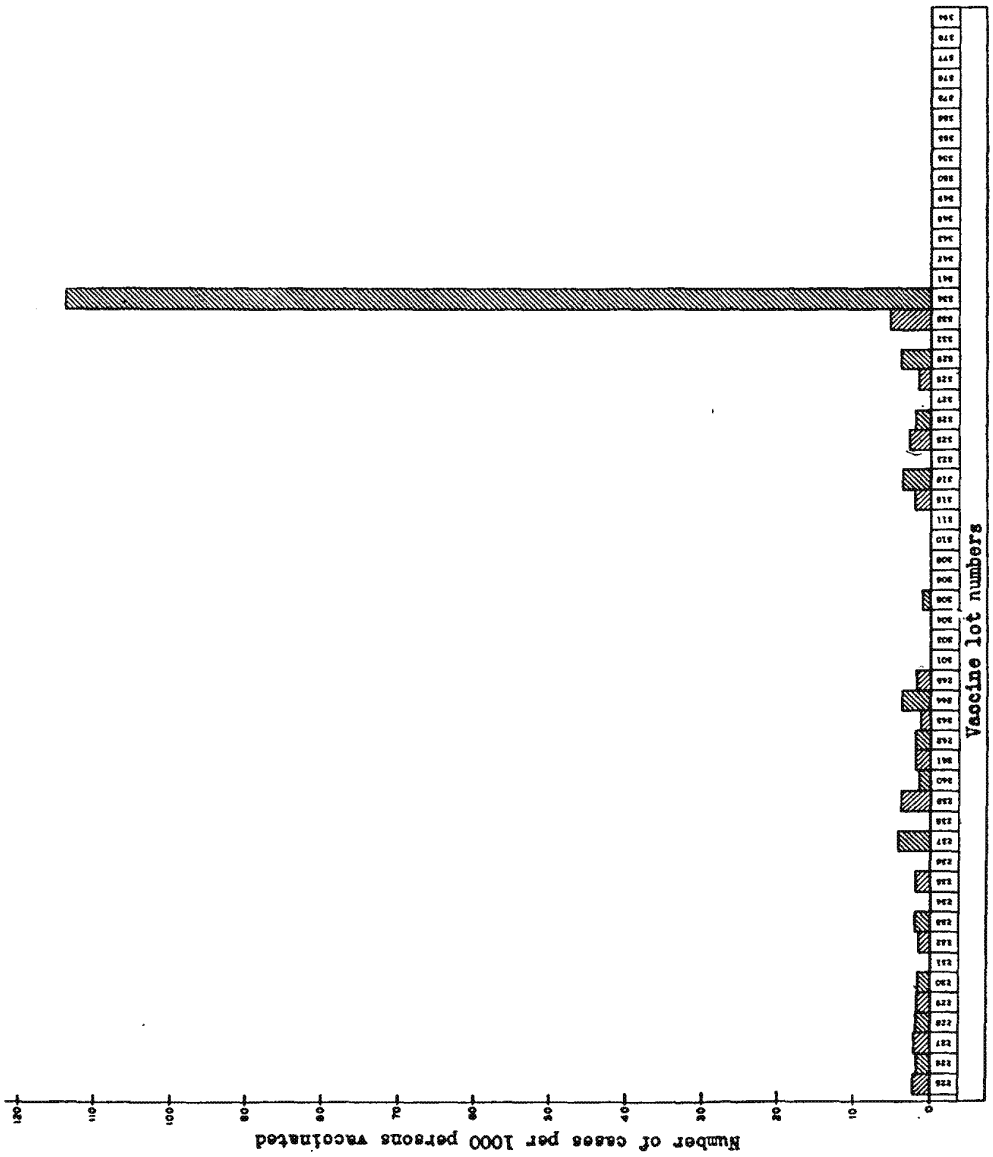

FIGURE 17. Postvaccination jaundice incidence in the United States Navy.

remarkable coincidence that all of the heavily icterogenic lots went principally to the Army. However, small amounts of some of the highly icterogenic lots were received by agencies other than the Army, and it is known that their use elsewhere was followed by cases of jaundice. Thus 12,000 doses from lot no. 331 were sent to the Virgin Islands for the immunization of civilians, and cases of jaundice following the use of this vaccine there were reported; 4,100 doses of lot no. 367 and 1,400 doses of lot no. 369 were sent to the Gold Coast, West Africa, but there is no information as to what was done with this material. No jaundice has been reported in any part of Africa except in persons vaccinated in the United States before their departure. Of lot no. 334, 8,000 doses were sent to Uganda, East Africa, but this material was not used, as a warning was sent from the laboratory that the lot appeared to be icterogenic.

G. Human serum as a possible source of the icterogenic agent

1. *Evidence available before the jaundice outbreak regarding possible danger from serum*

About the middle of March word was received from California of a sudden and widespread outbreak of jaundice in Army camps. As soon as the preliminary field investigations described in part I of this report indicated that certain lots of vaccine were apparently associated with the outbreak, the preparation of vaccine was suspended. As in Brazil and England earlier, suspicion at once fell on both the seed virus and the human serum as a possible source of the causative agent of the jaundice. The seed, however, was soon exonerated because the same seed was used to prepare all the lots of vaccine involved, while icterogenesis appeared to be associated only with a few of the lots. Therefore, all suspicion fell on the serum, and when vaccine production was later resumed, serum was omitted from both the seed virus and the vaccine.

The available earlier evidence suggesting that human serum may harbor the causal agent of the infective hepatitis was not at all convincing. Very little could be learned from the two outbreaks of jaundice which followed vaccination against yellow fever in Brazil. In the first of these episodes no conclusion was reached by those who investigated the incident except that the cases were limited to the recipients of two particular pools of hyperimmune monkey serum and that one of these pools was prepared by a person at a time when he suffered from an attack of catarrhal jaundice. The conclusions reached after the investigation of the second outbreak were likewise indefinite. The case incidence following the application of the same lot of vaccine varied greatly in different localities. The incidence was highest in places where a disease known commonly as catarrhal jaundice was endemic. Furthermore, the incidence increased with age. All this was taken as evidence against the infectious nature of the affliction, as otherwise one would expect different results, including immunity in older persons, especially in an endemic area. The workers concluded that the disease was probably of dual etiology, one factor being contained in the vaccine and the other contributed locally. No definite suspicion was cast upon human serum currently used in the vaccine, but more emphasis was laid on the possibility that the seed virus had become contaminated, and this largely because of the conclusions drawn by Findlay and his associates from their experience. Serum was eventually omitted from the

vaccine in Brazil, but the issue there at the time was further confused by the preliminary reports regarding the occurrence of postvaccination encephalitis which coincided with the omission of serum and which necessitated discontinuation of vaccination altogether for several months in 1941.

Findlay and his associates were the first to suggest (1) that postvaccination jaundice is of an infectious nature; (2) that it is probably due to a filterable virus; (3) that in their experience the icterogenic agent probably entered the seed virus cultures in human serum; and (4) that it was cultivated in series in symbiotic association with yellow fever virus. However, all blame was placed on the icterogenic contaminant in the seed virus cultures, and when the seed virus was changed, the use of human serum in the preparation of vaccine was continued. Reliance was placed upon the examination of blood donors and upon the inactivation of the serum. In fact, the use of human serum was not discontinued in England until January, 1943, when cases of jaundice began to occur among those vaccinated in October, 1942.

Except for the jaundice observed in England and Brazil in connection with yellow fever vaccination, there were only two early references in the literature suggesting that homologous serum may occasionally give rise to hepatitis in man. One of these was the experience of Propert (1938) with the use of measles convalescent serum. Seven children had each been given 4.5 ml of measles convalescent serum subcutaneously. About 3 months later all 7 developed severe icterus and 3 died. Mention of another similar experience was made by MacNalty (1938). About 100 children were given measles convalescent serum of the same pool. After a long incubation period 37 developed jaundice and 7 died.

However, measles convalescent serum has been used for prophylactic purposes on a very large scale both in England and in the United States without experiences similar to those referred to above having been reported. In fact, in a recent memorandum issued by the British Ministry of Health (1943) it is stated that during the last 10 years 366 liters obtained from more than 3,000 donors have been used in the London County Council Hospitals alone, and that the majority of the 36,000 recipients have been followed for 3 months or more without the detection of a case of jaundice among them. Moreover, there were no reports in the literature to indicate that delayed jaundice had been observed following the many thousands of blood transfusions that have been carried out every year throughout the world. There were, however, reports of negative results in attempts to transmit jaundice from man to man by means of direct blood inoculation. Thus Lainer (1940) reported his failure to transmit the disease by direct blood transfusion. He made 15 transfusions from typical cases of so-called catarrhal jaundice to normal persons, transferring 300 ml of blood in each instance, all with negative results. Transfers of duodenal content from cases of jaundice to normal individuals by means of a stomach tube likewise failed to produce the disease. In the latter experiments 300 ml of the material were transferred.

2. *Newer evidence to indicate the infectivity of human serum*

Since the investigative team began its work more evidence that serum from an apparently normal person may harbor the causal agent of infective hepatitis has been brought forward in publications

than was available earlier. A report of great importance covers the experience of Russian workers in immunization against pappataci fever. Although this report was actually published in 1940, unfortunately it did not come to the notice of the scientists in the United States especially interested in yellow fever prevention until the summer of 1942 when a copy of an English translation was very kindly furnished to the investigative team by Col. Bayne-Jones who received it from Lt. Col. Lucké. The article appeared in the *Terapevticheski Arkhiv*, but it was subsequently discovered that the number of the journal containing this particular report had not been received by any of the New York medical libraries, apparently because of war conditions.

This report, by Sergiev et al. (1940), describes an outbreak of jaundice which followed immunization against pappataci fever. In certain seasons of the year this fever is common in southern Russia along the Black Sea coast. Immunization consists of the injection of active pappataci virus and immune serum. In the summer of 1937, 500 persons were thus immunized in a particular locality without any ill effects. In the spring of 1939 immunization was again undertaken in the same locality. The inoculum consisted of human serum obtained from a person at the onset of experimental pappataci fever 3.5 days after the injection of a strain of the pappataci virus known as "A. Poliakova." The immune serum was obtained at a local blood transfusion station from donors who were known to have recovered recently from an attack of pappataci fever and were therefore immune. Each person received 0.01 ml of the virus-containing serum along with immune serum. The amount of immune serum given is not stated. Within the next 5 months there were 92 cases of jaundice among the 350 persons immunized, and at least one patient died. The incubation period in the majority was from 85 to 95 days. A single lot of virus serum and several batches of immune serum were used, and it was concluded that the virus serum contained the icterogenic agent. To obtain further proof, 0.1 ml of the virus-containing serum was injected into each of 4 mental patients in the Moscow Rural Psychiatric Hospital. One of these developed jaundice 4 months later. Fourteen other patients in the same institution inoculated similarly but with different samples of serum developed no jaundice.

A memorandum was published recently by the British Ministry of Health (1943) on the subject of homologous serum jaundice. In this memorandum additional information is given regarding the jaundice which followed the use of measles convalescent serum in England over 6 years ago. It appears that there were at least two icterogenic pools of measles convalescent serum used. One of these, known as K60, was prepared in April, 1936; it amounted to 880 ml and was derived from 26 measles convalescent donors. The pool was filtered, 0.5 per cent of preservative consisting of equal mixture of phenol and ether was added, and it was placed in 171 ampules. Of these, 121 were distributed between April, 1936, and June, 1937, in 9 localities in South and East England. It is established that at least 82 persons were certainly, and a further 27 probably, given injections from this pool. Of the total 109 recipients, 37 became ill with jaundice and 8 died. These include the cases reported by both Propert and MacNalty referred to earlier in this report, and it will be noted that the 7 children reported by Propert received their injection from this pool on June 1st, 1937,

which was over 13 months after the material was prepared. The second icterogenic pool was designated as K488. This was derived from adult donors, but other details, such as date of preparation and whether preservative was used, are not given. In one locality 58 ml were injected into 14 children; 6 of these subsequently developed jaundice and one died.

In the same memorandum mention is made of delayed jaundice following the injection of mumps convalescent serum. We are indebted to Dr. John E. Gordon for the following information based on the observations made by the staff members of the American Red Cross-Harvard Field Hospital Unit.

An outbreak of mumps began in November, 1941, in a local training station in England. Because of the continued prevalence of the disease and the high attack rate, it was decided to immunize passively those groups most heavily involved, using plasma from patients recently convalescent from mumps. In February, 1942, 11 volunteers, all recently recovered from mumps, were bled. Each donor contributed about 500 ml. The plasma was pooled, centrifuged and passed twice through Seitz filters. Merthiolate was added to a final concentration of 1 in 2,000. On March 12th, 266 volunteers were given 4, 5 or 6 ml of the merthiolated plasma intravenously. On March 21st, 11 other donors were similarly bled, and another pool of plasma was prepared; but to this no preservative was added. On March 28th, 214 men, all of whom had received their first inoculation from the merthiolated pool on March 12th, were each given 8 ml of the plasma from the second pool intravenously.

Jaundice was first noticed on May 26th, when 14 cases were reported among the inoculated groups. By then 101 of the original 266 had left the camp, but 79 (47.8 per cent) of the remaining 165 subsequently developed jaundice. There were 13 cases among the 26 who had received plasma only on March 12th and 66 cases among the 139 who had received injections from both of the plasma pools. The incubation period varied from 58 to 86 days, and there were no deaths.

The Ministry of Health memorandum referred to above also discusses the problem of jaundice following transfusion. A statement is made that recent investigations have brought to light a number of such cases, and particular reference is made to a group of 36 patients treated with massive transfusions of Seitz-filtered, pooled and dried serum for various forms of peripheral vascular disease. Of these, at least 8 were known to have developed jaundice. In discussing the whole problem the following comment is made: "It must, however, be remembered that no systematic follow-up of transfused patients had been attempted and that, since spontaneous recognition of an association between transfusion and late jaundice is unlikely to occur, it is not to be expected that such remote sequelae would be brought to notice of the transfusion officers. For this reason, it cannot be assumed that whole blood is innocent, or that plasma is likely to be less icterogenic than serum."

Among the still more recent reports of delayed jaundice following transfusion of serum or plasma are those of Beeson (1943) on a series of 7 cases in the United States and Morgan and Williamson (1943) on a similar series of 9 cases in England.

Of very great interest also is the experience recently reported by Cameron (1943), as it suggests a similarity between jaundice produced by injection of

blood or serum from persons with naturally acquired endemic jaundice and the postvaccination hepatitis observed elsewhere. Working in the Near East where a form of jaundice is known to be more or less endemic, Col. Cameron inoculated 7 volunteers intramuscularly with either 1 ml of serum or 2 ml of whole blood from spontaneously occurring cases of jaundice. Shortly after the inoculation one of the volunteers was sent off to field duty and therefore could not be followed. All the remaining 6 developed jaundice at varying intervals as follows:

One experienced a typical attack 32 days after inoculation. Blood and nasal washings from this patient were subinoculated into another volunteer. Up to 6 weeks after inoculation this volunteer had not developed jaundice; thereafter service exigencies precluded further observation. The remaining 5 of the volunteers originally inoculated were held under observation for 6 weeks, during which none developed jaundice. The results were considered negative and the men were assigned to various field units. However, all 5 subsequently developed jaundice within 6 months after inoculation.

Cameron's own opinion of the results of this experiment is that they are not so clear-cut as would be desired. He points out that there was some jaundice in the units in which the volunteers served, so that the possibility of infection from sources other than the original inoculation cannot be entirely excluded. He further points out, especially with reference to the very long incubation period in some of the volunteers, that the hepatitis virus apparently remained latent in some of them for a long time and developed into a frank hepatitis only after the resistance of the individual had been lowered as a result of strenuous service conditions entailing chills, fatigue and low rations; and had the men remained under more favorable conditions, they might not have developed hepatitis at all. Nevertheless, the appearance of jaundice in all of the 6 inoculated volunteers who were followed up, as compared with about 3 per cent in others, is very suggestive.

Referring specifically to the type of jaundice that has been observed to follow vaccination against yellow fever with certain lots of vaccine, Oliphant, Gilliam and Larson (1943) published results of their human transmission experiments. They succeeded in transmitting postvaccination jaundice to normal human volunteers by the subcutaneous injection of 0.15 ml of serum. The serum for transmission was obtained from persons with jaundice following routine large-scale vaccination as well as from persons who had been inoculated purposely with known icterogenic lots of vaccine. The average incubation period in the subinoculated individuals was about 12 weeks. The icterogenic agent was found in the circulating blood before jaundice appeared but not 2.5 months after recovery (one trial). The agent was found to pass Berkefeld N filters and to survive desiccation in vacuo and storage for long periods in serum at 4 C. It was inactivated by ultraviolet irradiation but not by heating for 30 minutes to 56 C in a dried state. All attempts to transmit the disease to laboratory animals failed. The authors came to the conclusion that the icterogenic agent was probably present in the human serum used in the preparation of yellow fever vaccine and that "there is an urgent need either for some means for detecting the presence of the jaundice-producing agent in the blood, or for some practical method for treating blood products so that the danger of jaundice following their use may be eliminated."

Findlay and Martin (1943) also have recently reported the transmission of jaundice following yellow fever vaccination to normal volunteers by intranasal instillation of nasal washings from persons with postvaccination jaundice.

In addition to the above, the following two unpublished reports suggest that serum from persons suffering from postvaccination jaundice may be infectious.

A technician, R. M. H., working in a military laboratory in California, developed jaundice late in October, 1942. She had not been vaccinated against yellow fever, but during the preceding 5 months she had done over 1,000 icterus index determinations on sera from patients suffering from postvaccination jaundice. It was learned that during the early part of August she had on two occasions accidentally sucked some of the serum into her mouth.

A physician in England, who had been vaccinated against yellow fever 6 months previously with a nonicterogenic lot, pricked his finger with a syringe needle while taking blood from a patient suffering from postvaccination jaundice. He developed an attack of hepatitis 4 months after the accident.

H. The nature of human serum used in the yellow fever vaccine

1. *Procedure for procuring human serum, and the extent of pooling*

Bleeding of donors was generally done once a week in Baltimore, usually on Tuesdays. Later in January and February, 1942, two bleedings were done in a week. Equipment and technique similar to that employed in bleeding persons for blood banks were used. The number of donors each week averaged about 30, but later this was increased to nearly 50. Blood was collected in 1-liter Erlenmeyer flasks and in the majority of instances was allowed to flow by gravity. On rare occasions suction was applied by a small hand-operated pump. As a rule, 400 to 500 ml were taken from each donor; the smaller amount was usually taken from women of slight build and weight as determined by the physician in charge.

The flasks with the blood were allowed to stand at room temperature for several hours, after which the serum was removed by suction through sterile rubber and glass tubing into a 2-liter flask, and each of the remaining clots was broken into 4 parts. The pooled serum in the 2-liter flasks was transferred to 250 ml centrifuge bottles and centrifuged for about 45 minutes at approximately 1,500 r.p.m. After centrifugation, the serum was transferred to sterile bottles holding 500 ml. After a sample for sterility test was removed, each bottle was sealed with a sterile rubber stopper for transportation to New York. The flasks containing the residual clots were placed in an icebox overnight, and on the following day the serum that had separated from the clot was drawn off and treated in the same manner as was the serum of the preceding day. In January, 1942, the technique was modified in that serum from the bleeding flasks was transferred directly into centrifuge bottles without intermediate pooling.

In New York a sterility test was done on the serum before and after inactivation. The manner in which the serum was handled in New York has already been described in connection with the vaccine preparation technique. It might be added that serum from a weekly shipment was used entirely at random, and bottles were labeled only for identification purposes in connection with sterility tests. The amount of serum necessary for each lot of vaccine was inactivated separately. There generally

was a tendency to overestimate the amount of serum needed, which usually resulted in a small surplus of inactivated serum. This surplus, which practically never amounted to more than one or two 100-ml flasks, was stored in the icebox and used in the next lot of vaccine.

As seen from the outline above, it is now entirely impossible to estimate how many donors actually contributed serum to a single lot of vaccine and in what proportion. In the preparation of a large lot, approximately 3,200 ml of serum were used, or approximately the amount obtained from 13 donors. In view of the procedures used, it would seem possible that, as a matter of chance, serum from an individual donor went into only a single lot of vaccine and was distributed among approximately 80,000 doses. On the other hand, it would seem equally possible that serum from a single donor could have been distributed in varying amounts among as many as 5 or 6 different lots, representing several hundred thousand doses. There was practically always a shortage of serum, and the amount of vaccine prepared was usually limited by the quantity of serum available. Thus serum received one week was usually all used up by the end of the following week, except perhaps a small carry-over which was insufficient even for a small lot of vaccine. For this reason, the lots of vaccine prepared with serum from any particular weekly bleeding can be traced with a fair degree of certainty.

2. *Blood donors*

In the preparation of the 141 lots of vaccine shown in table 33 over 311 liters of human serum were used. This amount was secured from about 970 different donors, a number of whom were bled more than once. As mentioned earlier in this report, the bleedings were done in the Department of Bacteriology, Johns Hopkins School of Hygiene and Public Health, by local personnel.

In May, 1942, when it appeared that at least 3 lots, no. 331, 335 and 338, were associated with an abnormally high incidence of jaundice, and when the use of serum in the vaccine had been discontinued, Drs. Kenneth F. Maxcy and Ross L. Gauld, both of the Johns Hopkins School of Hygiene and Public Health, undertook to investigate the donors who had been bled in 1941. Inasmuch as a large proportion of the donors were medical students, and as the summer recess was at hand, not all donors could be investigated. However, questionnaires were returned by 396. It was learned that 7 of the donors had suffered from an attack of jaundice 1 to 20 years previously. In one instance jaundice associated with malaria occurred only a few months before the donor was bled. However, none of the vaccine lots in which the serum of these donors was used appeared to be associated with jaundice. On the other hand, donors who had contributed to the frankly icterogenic lots gave no history of jaundice, although a number of them had suffered from gastrointestinal disturbances of various forms either immediately before or shortly after bleeding. The investigators also noted that about 5 times as many patients with history of jaundice were discharged from the Johns Hopkins Hospital during the latter half of 1941 than in the first half of the same year.

Later in 1942 when it became obvious that additional lots of vaccine, especially those prepared early in 1942, such as no. 367, 368 and 369, were associated with a very high incidence of jaundice, Col. Bayne-Jones, and Maj. Walker undertook a systematic investigation of all blood donors whose serum had been

used in the preparation of the vaccine. Unfortunately, many of the donors could no longer be reached, but histories were obtained from a total of 367. These included some who already had been studied in May, while a considerable amount of new information was obtained from donors who had been bled early in 1942 and who had not been included in the study made in May.

It is with the kind permission of Col. Bayne-Jones and Maj. Walker that some of their findings are included in this report. They are summarized in table 38. For the purposes of analysis, serum from a weekly bleeding was given a lot letter or number, and the table shows which lots of vaccine were made with a given lot of serum. It also shows in which vaccine lots serum from two bleedings was mixed. The degree of icterogenesis for the incriminated lots is designated by plus signs. Lots that showed a case rate between 1 and 5 per 1,000 doses of vaccine supplied to the Army, or between 2 and 5 per 1,000 persons vaccinated in the Navy, are indicated by +; these lots are considered mildly icterogenic. Lots which had a case rate between 5 and 10 per 1,000 doses supplied to the Army, or the same rate per 1,000 persons vaccinated in the Navy are marked ++; these lots are considered moderately but definitely icterogenic. All highly icterogenic lots with a case rate over 10 per 1,000 are marked +++. In table 38 are also shown the number of persons bled for each serum lot, the date of bleeding, and number of donors subsequently investigated, the number giving history of jaundice, and the date and type of attack of jaundice. It will be noted that there were 23 donors with a history of jaundice, with the time of attack varying from early infancy to a few weeks before bleeding in one instance.

In tables 39, 40 and 41 is presented a

TABLE 38

Donors of serum used in yellow fever vaccine, with special reference to history of jaundice

Date of bleeding	Donors bled	Histories obtained	Donors with history of jaundice	Serial number and name (initials) of donors, with history of jaundice, type of jaundice and date of attack	Serum lot designation	Vaccine lot number	Icterogenic property of lot*
Jan. 16, 1941	23	8	1	No. 17, L. E. Catarrhal jaundice in 1938	C	206	
Feb. 11, 1941	22	10			F	212	
Mar. 4, 1941	22	8			I	217	
Mar. 10, 1941	22	5			K	219	
					K	220	
Mar. 17, 1941	23	0			L	221	
					L	222	
					L	223	
Mar. 25, 1941	25	25	2	No. 15, J. A. D. Catarrhal jaundice in 1931	M	224	
				No. 16, A. T. Catarrhal jaundice in 1934	M	225	+
Apr. 1, 1941	27	27	2	No. 9, A. B. Jaundice in 1921, type not known	N	226	
				No. 21, S. R. Catarrhal jaundice in Nov. 1940	N	227	
					N	228	
					N+O	229	+
Apr. 8, 1941	28	28	3	No. 4, C. C. Jaundice in childhood, type not known	O	230	
				No. 10, J. G. M. Jaundice in 1924, type not known	O	231	
				No. 19, H. T. Catarrhal jaundice in 1939	O	232	

TABLE 38—*Continued*

Date of bleeding	Donors bled	Histories obtained	Donors with history of jaundice	Serial number and name (initials) of donors, with history of jaundice, type of jaundice and date of attack	Serum lot designation	Vaccine lot number	Icterogenic property of lot*
Apr. 15, 1941	30	30	2	No. 8, L. B. Catarrhal jaundice in 1914	P	233	+
				No. 14, J. H. H. Catarrhal jaundice in 1930	P	234	
					P	235	
					P	236	
					P	237	+
					P	238	
					P	239	+
					P+R	240	
Apr. 22, 1941	27	27	3	No. 5, F. R. Catarrhal jaundice in childhood	R	241	
				No. 11, R. A. L. Catarrhal jaundice in 1925	R	242	
				No. 12, G. M. Catarrhal jaundice in 1926	R	243	
					R	244	+
					R	245	
					1†	301	
					1	303	
					1	304	
					2+1	305	
Sept. 3, 1941	31	30	1	No. 16, A. T. Catarrhal jaundice in 1934	2	306	+
					2+3	307	+++
Sept. 9, 1941	31	30			3	308	
					3	310	+
Sept. 15, 1941	32	32			4	311	+
					4	312	+
					4	313	
					4	315	
Jan. 27, 1942	43	33	1	No. 23, J. F. Catarrhal jaundice in 1936; second attack in Dec., 1941	19	363	+
					19	364	+
					19	365	+
					19	366	+
					19+20	367	+++
Jan. 30, 1942	24	20	1	No. 22, J. H. M. Catarrhal jaundice in Feb., 1941	20	368	+++
					21+20	369	+++
Feb. 3, 1942	46	31	2	No. 2, E. B. Jaundice in infancy, type not known	21	370	++
				No. 6, E. L. Z. Catarrhal jaundice in 1903	21	371	
					21+22	372	+
Feb. 6, 1942	26	6			22	373	+
					23+22	374	
Feb. 10, 1942	45	12	1	No. 17, L. E. Catarrhal jaundice in 1938	23	375	
					23	376	
					23+24	377	
Feb. 13, 1942	31	1			24	378	
					24	379	
					25+24	380	
Feb. 17, 1942	39	3			25	381	
					25	382	
					25+26a	383	
Feb. 20, 1942	36	1	1	No. 16. A. T. Catarrhal jaundice in 1934	26a	384	
					26a	385	
					26b+26a	386	
Feb. 24, 1942	44	5	2	No. 7, H. N. H. Catarrhal jaundice in 1912	26b	387	
				No. 18, K. G. Malarial jaundice in 1939	26b	388	
					26b	389	
					26b+27	390	+
Feb. 27, 1942	37	0			27	391	
					27	392	
					28+27	393	
Mar. 3, 1942	41	5	2	No. 1, K. S. Icterus neonatorum	28	394	
				No. 20, R. M. R. Catarrhal jaundice in 1939	28	395	
					28	396	
					28+29a	397	
Mar. 6, 1942	35	1			29a	398	

TABLE 38—*Continued*

Date of bleeding	Donors bled	Histories obtained	Donors with history of jaundice	Serial number and name (initials) of donors, with history of jaundice, type of jaundice and date of attack	Serum lot designation	Vaccine lot number	Icterogenic property of lot*
Mar. 10, 1942	40	1			29b+29a	399	
Sept. 22, 1941	31	27			5	316	+
					5	317	++
					5	318	+
					5+6	319	++
Sept. 29, 1941	30	30			6	320	+
					6	321	
					7+6	322	+
Oct. 6, 1941	30	28			7	323	
					7	324	+
					7+8	325	+
Oct. 13, 1941	29	29			8	326	
					8	327	+
					8	328	
					9+8	329	+++
Oct. 20, 1941	30	30	1	No. 1, K. S. Icterus neonatorum	9	330	+
					9+10	331	+++
Oct. 27, 1941	30	29	2	No. 7, H. N. H. Catarrhal jaundice in 1912	10	332	+
				No. 8, L. B. Catarrhal jaundice in 1914	10	333	++
					10	334	+++
Nov. 3, 1941	29	29	4	No. 17, L. E. Catarrhal jaundice in 1938 No. 18, K. G. Malarial jaundice in 1939 No. 20, R. M. R. Catarrhal jaundice in Dec., 1939 No. 22, J. H. M. Catarrhal jaundice in Feb., 1941	11+10	335	+++
Nov. 10, 1941	30	26	1	No. 3, W. B. Catarrhal jaundice in childhood	12	338	+++
Nov. 19, 1941	30	25			13	340	+
					13+14	341	+
Nov. 25, 1941	29	25	1	No. 13, ? Jaundice in 1926, type not known	14	342	
					14	343	
					14	344	+
					15+14	345	+
Dec. 2, 1941	29	26	1	No. 16, A. T. Catarrhal jaundice in 1934	15	346	+
					15	347	
					15	348	
					15	349	++
Jan. 6, 1942	39	16			16	350	+
					16	351	+
					16	352	
Jan. 12, 1942	51	23			17	353	
Jan. 13					17	354	
					17	355	++
					17	356	++
					17	357	+
					17+18	358	
Jan. 20, 1942	49	23			18	359	
					18	360	+
					18	361	
					18	362	+

* + Slightly or moderately icterogenic; ++ definitely icterogenic; +++ highly icterogenic.

† Same as serum lot R, except it was held in storage for 4 months and passed through a Berkefeld filter before being used.

further break-down of the data shown in table 38. In table 39 the donors with a history of jaundice are arranged chronologically according to the date of their attack of jaundice. The dates of bleeding and the serum lots to which each con-

tributed are also shown, as are the icterogenic vaccine lots made with these serum lots. It will be noted that 6 of the 23 donors with a history of jaundice were bled twice; one was bled 3 times, and one 4 times. It will also be noted that each donor with a history of jaundice contributed to an icterogenic lot of vaccine, although not on each bleeding.

Table 40 lists 20 serum lots which contained serum from donors with a history of jaundice. It will be noted that among them are 4 serum lots, C, 23, 26a and 28, which were used for the preparation of vaccine lots that did not prove to be icterogenic, although the same donors had on other occasions contributed to other lots which were associated with jaundice.

In table 41 are shown 8 lots of serum from donors without a history of jaundice. Eighteen lots of vaccine made from this serum were moderately icterogenic.

In studying the tables above, it must be borne in mind that a serum lot was not in a strict sense a pool but had been mixed to an indeterminable degree. As

TABLE 39

Serum donors with history of jaundice arranged chronologically according to date of attack, date of bleeding and vaccine lots in which their serum was used

Number and initials of donor	Attack of jaundice		Date of bleeding	Serum lot designation	Lot numbers of icterogenic vaccine in which serum was used	
	Date	Type			Highly icterogenic	Moderately icterogenic
1. GRO-A	Infancy	Icterus neonatorum	Oct. 20, 1941 Mar. 3, 1942	9 28	329, 331	330
2. GRO-A	Infancy	Not stated	Feb. 3, 1942	21	369	370
3. GRO-A	Childhood	Catarrhal?	Nov. 10, 1941	12	338	
4. GRO-A	Childhood	Not stated	Apr. 8, 1941	0		229
5. GRO-A	Childhood	Catarrhal	Apr. 22, 1941	R		244
6. GRO-A	1903	Catarrhal	Feb. 3, 1942	21	369	370
7. GRO-A	1912	Catarrhal	Oct. 27, 1941 Feb. 24, 1942	10 26b	331, 334, 335	332, 333 390
8. GRO-A	1914	Catarrhal	Apr. 15, 1941 Oct. 27, 1941	P 10	 331, 334, 335	233, 237, 239 332, 333
9. GRO-A	1921	Not stated	Apr. 1, 1941	N		229
10. GRO-A	1924	Not stated	Apr. 8, 1941	0		229
11. GRO-A	1925	Catarrhal	Apr. 22, 1941	R		244
12. GRO-A	1926	Catarrhal	Apr. 22, 1941	R		244
13. ?	1926	Not stated	Nov. 25, 1941	14		341, 344

TABLE 39—*Continued*

Number and initials of donor	Attack of jaundice		Date of bleeding	Serum lot designation	Lot numbers of icterogenic vaccine in which serum was used	
	Date	Type			Highly icterogenic	Moderately icterogenic
14. GRO-A	1930	Catarrhal	Apr. 15, 1941	P		233, 237, 239
15. GRO-A	1931	Catarrhal	Mar. 25, 1941	M		225
16. GRO-A	1934	Catarrhal	Mar. 25, 1941 Sept. 3, 1941 Dec. 2, 1941 Feb. 20, 1942	M 2 15 26a	 307	225 306 345, 346, 349
17. GRO-A	1938	Catarrhal	Jan. 16, 1941 Nov. 3, 1941 Feb. 10, 1942	C 11 23	 335	
18. GRO-A	1939	Malarial	Nov. 3, 1941 Feb. 24, 1942	11 26b	335	 390
19. GRO-A	1939	Catarrhal	Apr. 8, 1941	0		229
20. GRO-A	Dec., 1939	Catarrhal	Nov. 3, 1941 Mar. 3, 1942	11 28	335	
21. GRO-A	Nov., 1940	Catarrhal	Apr. 1, 1941	N		229
22. GRO-A	Feb., 1941	Catarrhal	Nov. 3, 1941 Jan. 30, 1942	11 20	335 367, 368, 369	
23. GRO-A	1936 Dec., 1941	Catarrhal Catarrhal	Jan. 27, 1942	19	367	363, 364, 365, 366

pointed out earlier in this report, it seems entirely possible that, as a matter of chance, serum in some of the lots became fairly well mixed during the separation and later handling. On the other hand, there seems to be an equal chance that only a limited degree of mixing occurred, and that serum from an individual donor as a rule went into only a single lot of vaccine.

An analysis of the data presented in these tables reveals the fact that donors with a history of a relatively recent attack of jaundice contributed serum to the weekly shipment which was used in the preparation of most of the highly icterogenic lots of vaccine.

Beginning with the most icterogenic lots of all, no. 367, 368 and 369, it will be noted that serum from 24 donors was used in the preparation of these lots. Subsequently only 20 of the 24 donors could be interrogated, and only one gave a positive history of jaundice. This donor, GRO-A (no. 22), had an attack of catarrhal jaundice in February, 1941. On November 3rd, i.e., approximately 9 months after his illness, he was bled and his serum was used along with that from other donors, 3 of whom had a history of jaundice, in the preparation of vaccine

lots no. 335, 336 and 337. Lot 335 proved highly icterogenic. Lots 336 and 337 were discarded because of bacterial contamination, and their icterogenic property is not known. On January 30th, or about 1 year after his attack of jaundice, GRO-A. was bled again along with 23 others, and the serum from that bleeding went into lots no. 367, 368 and 369, the most icterogenic of all lots. Since GRO-A was the only donor in this group having a positive history of previous jaundice, it would seem reasonable to suspect that the icterogenic agent in these lots originated from the blood of this donor.

Another donor of considerable interest is GRO-A, no. 23, whose serum was in the bleeding of January 27th, 1942, which had been used in the moderately icterogenic lots no. 363, 364, 365 and 366; a small residue may also have gone into 367. Only 33 of the 43 donors bled on that date could subsequently be investigated, and the past history of the remaining 10 is not known. However,

TABLE 40

Relationship of serum lots containing serum from donors with history of jaundice to icterogenesis of vaccine lots in which the serum was used

Serum lot	Date of bleeding	Donors with history of jaundice	Lot numbers of icterogenic vaccine in which the serum was used	
			Highly icterogenic	Moderately icterogenic
C	Jan. 16, 1941	No. 17, L. E., Catarrhal jaundice in 1938		
M	Mar. 25, 1941	No. 15, J. A. D., Catarrhal jaundice in 1931 No. 16, A. T., Catarrhal jaundice in 1934		225
N	Apr. 1, 1941	No. 9, A. B., Jaundice in 1921, type? No. 21, S. R., Catarrhal jaundice Nov. 1940		229
0	Apr. 8, 1941	No. 4, C. C., Jaundice in childhood No. 10, J. G. M., Jaundice in 1924, type? No. 19, H. T., Catarrhal jaundice in 1939		229
P	Apr. 15, 1941	No. 8, L. B., Catarrhal jaundice in 1914 No. 14, J. H. H., Catarrhal jaundice in 1930		233, 237, 239
R	Apr. 22, 1941	No. 5, F. R., Jaundice in childhood No. 11, R. A. L., Catarrhal jaundice in 1925 No. 12, G. M., Catarrhal jaundice in 1926		244
2	Sept. 3, 1941	No. 16, A. T., Catarrhal jaundice in 1934	307	306
9	Oct. 20, 1941	No. 1, K. S., Icterus neonatorum	329, 331	330
10	Oct. 27, 1941	No. 7, H. N. H., Catarrhal jaundice in 1912 No. 8, L. B., Catarrhal jaundice in 1914	331, 334, 335	332, 333
11	Nov. 3, 1941	No. 17, L. E., Catarrhal jaundice in 1938 No. 18, K. G., Malarial jaundice in 1939 No. 20, R. M. R., Catarrhal jaundice, Dec. 1939 No. 22, J. H. M., Catarrhal jaundice, Feb. 1941	335	

TABLE 40—*Continued*

Serum lot	Date of bleeding	Donors with history of jaundice	Lot numbers of icterogenic vaccine in which the serum was used	
			Highly icterogenic	Moderately icterogenic
12	Nov. 10, 1941	No. 3, W. B., Jaundice in childhood	338	
14	Nov. 25, 1941	No. 13, ?, Jaundice in 1926, type?		341, 344, 345
15	Dec. 2, 1941	No. 16, A. T., Catarrhal jaundice in 1934		345, 346, 349
19	Jan. 27, 1942	No. 23, J. F., Catarrhal jaundice in 1936; second attack in Dec. 1941	367	363, 364, 365, 366
20	Jan. 30, 1942	No. 22, J. H. M., Catarrhal jaundice, Feb. 1941	367, 368, 369	
21	Feb. 3, 1942	No. 2, E. B., Jaundice in infancy No. 6, E. L. Z., Catarrhal jaundice in 1903	369	370, 372
23	Feb. 10, 1942	No. 17, L. E., Catarrhal jaundice in 1938		
26a	Feb. 20, 1942	No. 16, A. T., Catarrhal jaundice in 1934		
26b	Feb. 24, 1942	No. 7, H. N. H., Catarrhal jaundice in 1912 No. 18, K. G., Malarial jaundice in 1939		390
28	Mar. 3, 1942	No. 1, K. S., Icterus neonatorum No. 20, R. M. R., Catarrhal jaundice in 1939		

GRO-A. is of interest because he is stated to have been convalescing from an attack of catarrhal jaundice when bled on January 27th, and it is surprising that his condition was not detected at the time. It seems that this donor, who incidentally is a physician, had an attack of so-called catarrhal jaundice in 1936. On December 5th, 1941, he was again hospitalized with another attack of the same disease and discharged on December 18th, 1941. According to his own statement, his sclerae were still icteric when he contributed his blood about a month later.

Into the preparation of the icterogenic lot no. 335 went serum from 29 donors, all of whom were available for subsequent investigation. In addition to donor GRO-A., no. 22, discussed above in connection with lots no. 367, 368 and 369, there were 3 donors with recent history of jaundice. They were GRO-A, GRO-A., and GRO-A., nos. 17, 19 and 20, respectively. GRO-A. was said to have suffered from an attack of catarrhal jaundice in 1938; GRO-A became jaundiced during an attack of malaria in 1939; and GRO-A. had an attack of catarrhal jaundice also in 1939.

With regard to icterogenic lot no. 338, serum from 30 donors was used in the preparation of this and lot no. 339 which was discarded because of bacterial contamination. Only 26 of the donors were available for later investigation, and among them there was only one who gave a history of previous jaundice. This donor, GRO-A, no. 3, was stated to have had an attack of jaundice in childhood.

In the preparation of icterogenic lots

no. 331, 332, 333 (moderately icterogenic in the Navy), and 334, serum from 30 donors was used, and of these 29 were investigated. There were two donors among them with a previous history of catarrhal jaundice. One of these, GRO-A, no. 7, had the disease in 1912, and the other, GRO-A no. 8, in 1914, or 29 and 27 years, respectively, before their serum was used in the vaccine.

All donors who contributed to the icterogenic vaccine lots no. 329 and 330 were available for subsequent study, and among them was only one with a history of jaundice. This donor, GRO-A, no. 1, was stated to have suffered from an attack of icterus neonatorum.

Donors who contributed to icterogenic lot no. 307 gave no history of jaundice, but donor GRO-A, no. 16, whose serum went into lots no. 305 and 306, and a residue of which may also have gone into lot no. 307, gave a history of an attack of catarrhal jaundice in 1934.

This establishes an apparent relationship between all of the highly icterogenic lots of vaccine included in table 35 and a history of an attack of jaundice at least in some of the donors whose serum was used in the preparation of these lots. However, there still remain 18 moderately icterogenic lots of vaccine included in table 38, and listed separately in table 41, which were prepared from serum of donors (most of whom were available for subsequent study) who gave no history of jaundice.

TABLE 41

Moderately icterogenic lots of vaccine prepared with serum from donors who gave no history of jaundice

Serum lot	Date of bleeding	Donors bled	Number subsequently investigated	Vaccine lot numbers	Jaundice incidence per 1,000 doses
	(1941)				
3	Sept. 9	31	30	310	1.09
4	Sept. 15	32	32	311	1.65
				312	2.36
5	Sept. 22	31	27	316	3.50
				317	5.49
				318	2.04
				319	9.38
6	Sept. 29	30	30	320	2.25
7	Oct. 6	30	28	322	4.44
				324	4.81
				325	1.12
	(1942)				
16	Jan. 6	39	16	350	4.78
				351	2.59
17	Jan. 13	51	23	355	5.41
				356	5.00
				357	1.92
18	Jan. 20	49	23	360	2.36
				362	1.39

3. *General comment*

As the detailed histories of nearly two-thirds of all blood donors are still missing, it is exceedingly difficult to arrive at any definite conclusion as to the significance of the donors' positive jaundice histories in relation to the icterogenesis of the vaccine lots which contained serum from such donors. Any conclusion drawn on the basis of the present available information is bound to be more or less speculative, as it will be necessary to make several important assumptions. Nevertheless, the material presented above seems highly suggestive.

The basic fact is brought out that all highly icterogenic lots of vaccine apparently contained a certain amount of serum from persons who had suffered from an attack of jaundice sometime in the past. In some instances, as with donors GRO-A (no. 3) and GRO-A (no. 1), whose serum went into icterogenic vaccine lots no. 329, 330 and 331 in the case of GRO-A, and no. 338 in the case of GRO-A the attack dates back to infancy or early childhood. In others, especially

those who contributed to the other highly icterogenic lots, the attack of jaundice occurred much more recently. There are very few lots of vaccine which contained serum from donors with a history of previous jaundice without showing suspected icterogenic tendencies on the basis of information at present available.

If the icterogenic agent in the vaccine originated from donors who had suffered an attack of jaundice previously, then it would seem that such persons had become more or less permanent carriers of the agent. In the study reported above, 23 (6.3 per cent) of 367 donors, gave a positive history of jaundice. Under this supposition about 6 per cent of a normal population might have been periodic carriers of the icterogenic agent. In table 36 are shown 38 suspected moderately icterogenic lots of vaccine used in the Army, and there are 5 additional lots of similar category used in the Navy. The donors whose serum was used in the preparation of a considerable number of these lots have given no history of jaundice. If the icterogenesis of these lots also originated from human serum, there must have been a considerable number of additional carriers who gave no history of previous jaundice, and on this basis the carrier rate in the normal population might be considerably higher than 6 per cent.

There is no evidence from the blood transfusion experience or from the wise use of convalescent sera that such a proportion of carriers actually exists. But, on the other hand, it is difficult to visualize a more sensitive test for the icterogenic properties of human serum than the procedure used for vaccination against yellow fever. In transfusion, even with pooled plasma from blood banks, plasma from a single donor in a pool probably never is divided among more than 25 recipients, and if some of these recipients should subsequently develop jaundice many weeks later, the event probably would not be attributed to the possible icterogenesis of the transfused plasma. In yellow fever vaccination, serum from a single donor was injected into a very large number of recipients, and if such serum contained an icterogenic agent, the aftereffect assumed major epidemic characteristics and made itself obvious. This of course applies only to frankly icterogenic lots of vaccine; other lots that possessed only slight or moderate degree of icterogenesis might not be so easily detected.

If human serum used in the preparation of yellow fever vaccine was the sole source of the agent responsible for the postvaccination hepatitis, it would be apparent that at times this agent must be present in human serum in an extremely high concentration. One immunizing dose of vaccine contained approximately 0.04 ml of human serum and 0.01 ml of chick juice. In the preparation of a large lot of vaccine, such as no. 367, 368 and 369, the amount of serum used in each lot represented the equivalent of that obtained from about 13 donors. If, by chance, serum from only one carrier donor went into a single lot of vaccine of about 80,000 doses, the maximum amount of serum from this one donor among 13 in an immunizing dose could not exceed 0.0032 ml. Vaccine lots no. 367, 368 and 369 were all highly icterogenic. This suggests that some material containing the icterogenic agent was fairly evenly distributed among these lots. The serum from the bleeding done on January 30th was the only common constituent of these lots; everything else went separately into each individual lot. There were 24 donors bled on that date. In addition, lot no. 367 also received some serum obtained in

the preceding bleeding, and to lot no. 369 was added serum from bleeding done on February 3rd. As mentioned above, 20 of the 24 donors were available for investigation, and among them was one, GRO-A, who a year previously had suffered from an attack of catarrhal jaundice. It would therefore seem reasonable to suspect that the serum from this donor was the sole source of the icterogenic agent introduced into these lots and that it was more or less evenly distributed among them. These 3 lots together contained slightly more than 240,000 doses. The amount of serum obtained from GRO-A probably amounted to about 250 ml. This would mean that his serum must have contained enough of the icterogenic agent to be infective in doses of about 0.001 ml. This would seem an incredible concentration, especially 1 year after the attack of the disease, and would suggest that there are no neutralizing antibodies developed in this disease, unless their effect was suppressed by dilution. Conditions somewhat similar are known to occur in certain virus diseases as, for example, in infectious anemia of horses, where virus has been shown to be present in the blood many years after the recovery from an attack (Schalk and Roderick, 1923; Heath, 1931).

The experience of the Russian investigators already referred to, in their vaccination against pappataci fever, seems to be the only example described in the medical literature that is somewhat comparable to the experience with yellow fever vaccine. They demonstrated that 0.01 ml of icterogenic serum, which also contained pappataci virus, produced hepatitis in 26 per cent of those inoculated. Smaller amounts were not tested, but it was shown that 10 times larger doses, i.e., 0.1 ml, produced jaundice in only 1 of 4 tested. In view of the high proportion infected by 0.01 ml, it would be reasonable to assume that smaller amounts would have been infective. All other reports in the literature describing jaundice following the injection of homologous serum deal with relatively large amounts injected, and protective antibodies may have been present in effective quantity. In fact, in a recent editorial in Lancet (1943) it was pointed out that, on the evidence available at present, small doses of homologous serum seem more effective in producing hepatitis in man than large volumes. For this reason, much more concrete information must become available before any definite conclusion can be drawn regarding the nature of the etiological agent of the postvaccination jaundice and the role played by human serum in the pathogenesis of this disease.

I. Possibility of seed virus contamination

If the apparent icterogenesis had been limited to only a few lots of vaccine, the explanation that the agent was introduced by way of serum from human carriers who had suffered from an attack of jaundice would perhaps have been satisfactory. But, in view of the fact that at least 40 per cent of all lots used in the Army, in addition to several used in the Navy, appear to have been associated with varying amounts of jaundice, it becomes increasingly difficult to explain the icterogenesis of all these lots on the basis of human serum alone, unless there was an epidemic of inapparent infection which lasted over a long time among the blood donors in Baltimore. It is obviously possible that much of this jaundice in the armed forces may have been due to ordinary "catarrhal" jaundice independent of vaccination or to error in the recording of

lot numbers, but this would not seem to explain it all. As was pointed out earlier, no evidence has as yet come to light which would indicate that carriers of the icterogenic agent are very common in a normal population. Therefore, additional sources for the causative agent of the infective hepatitis must be considered. Here the seed virus naturally comes under suspicion. It is true that more than 50 per cent of the vaccine lots made with the same seed virus apparently possessed little or no icterogenic properties. Furthermore, as seen from table 33, 147,500 doses of the same seed virus no. 147–1 was sent to Africa and used there as a vaccine without postvaccination icterus being observed. All this seems to exonerate the seed virus. However, it must be borne in mind that the etiological agent of the hepatitis is still unknown, and from the fragmentary information available in the literature it seems to possess some very peculiar properties. Until more information becomes available, the possibility that the agent may remain quiescent in the seed virus for long periods cannot be entirely ignored.

Clinically the hepatitis in man observed after the injection of human blood products in connection with various immunization procedures appears uniform in its manifestations. The fatal cases apparently are also histopathologically indistinguishable, regardless of whether death followed the use of yellow fever vaccine, measles convalescent serum, or spontaneously acquired disease. Therefore, it would seem reasonable to assume that the hepatitis, however acquired, is the same disease entity and caused by the same etiological agent. Findlay and his associates, as well as the Russian investigators, and more recently Cameron, already referred to, have come to a firm conclusion that the causative agent of the hepatitis is a filterable virus. On the assumption that they are correct in their conclusion, this agent must be a virus with some very unusual characteristics. It obviously passes Seitz and Berkefeld filters with ease. It must have a greater thermal stability range than most other known viruses that are pathogenic to man and apparently survives heating to 56 C for at least 30 minutes. It must survive desiccation as applied in the processing of human serum and plasma for transfusion. It must survive for more than 1 year in liquid serum containing 0.25 per cent of phenol, as in batch K60 of measles serum reported in the British Ministry of Health memorandum. In the human host it apparently behaves in a most unusual manner, as manifested by the extraordinary range in the incubation period, varying anywhere from less than 1 month to nearly 1 year. It must have a tendency to localize selectively in the liver and to give rise to a hepatitis syndrome in only a certain proportion of individuals, while in the others it evidently appears in the circulating blood in a relatively high concentration without causing any ill effects whatsoever.

There are, of course, some viruses that are known to possess extraordinary resistance to heat and chemical disinfectants. The virus of the infectious anemia of horses (swamp fever) has been reported to withstand heating for 1 hour at 58 C and the effect of 0.1 per cent phenol for 8 weeks (Heiermann, 1923). The virus of African horse sickness has been reported to withstand 3 per cent phenol for a long period of time, and 0.25 per cent is used routinely as a preservative for material containing this virus, but its thermal stability is reported to be slight (Theiler, 1930). The virus of fowl plague has also been reported to withstand heating for 30 minutes at 60 C

without loss of virulence. Long incubation periods are also known for other virus diseases, such as rabies, for example, and especially fowl paralysis, which by some is believed to be caused by a virus.

There is as yet no definite evidence to indicate that the etiological agent of hepatitis can be cultivated either in tissue culture or in developing chick embryo. It is true that contamination of yellow fever seed virus with the agent of hepatitis was suspected both in Brazil and in England. It must be remembered, however, that these vaccines all contained a certain amount of human serum which may have served directly as a primary source for the agent of hepatitis. If the virus nature of the agent is recognized, then the possibility of its cultivation in tissue cultures and in chick embryos must also be admitted. And if such a possibility is admitted, there will appear an element of danger in all present-day yellow fever vaccination procedures regardless of whether human serum is used in the preparation of the vaccine, unless the seed virus is actually entirely free of the icterogenic agent.

It was stated earlier in this report that the 17D virus has had a long association with human serum during the period of cultivation in vitro in tissue culture. Therefore, the possibility that the agent of hepatitis may have been introduced repeatedly into the early tissue cultures must be recognized. Moreover, if the agent should be capable of propagation under the same conditions as those under which yellow fever virus was cultivated, it would seem possible that all existing branches of the original 17E and 17D virus might be contaminated. This naturally would include all known variants of modified yellow fever virus that are suitable for human immunization.

During the in vitro cultivation of yellow fever virus subcultures were generally made at 4-day intervals. Embryos inoculated with the seed virus for yellow fever vaccine are likewise incubated for 4 days before they are harvested. If the very long incubation period in man is taken as an indication of a slow multiplication rate for the agent of hepatitis in general, much multiplication could not be expected either in tissue cultures or in chick embryos during the 4 days of incubation, which in itself might explain the apparent absence of icterogenic properties in most of the vaccine lots. However, this will not rule out the possibility that conditions in some individual embryos may be more favorable for the multiplication of the agent than in others. In the preparation of a large lot of vaccine consisting of about 80,000 doses, approximately 250 embryos were used. Each embryo must be considered as an individual living animal, and it is known that individual embryos vary in their susceptibility to a number of viruses. Thus in a given group of embryos of equal age, inoculated at the same time and with identical amounts of yellow fever virus, a wide variation in mortality rate and in virus concentration has frequently been observed. If the agent of hepatitis should behave in a somewhat similar manner, then it would seem perfectly logical to expect that in occasional embryos it might find exceptionally favorable conditions for multiplication, and that in such instances it might attain a sufficiently high concentration to render the entire vaccine lot icterogenic. The possibility of such a moderate multiplication of the agent of hepatitis in an occasional embryo might perhaps explain the low degree of icterogenesis of a large number of vaccine lots. It will be recalled that in a similar outbreak of

postvaccination jaundice in Brazil reported by Fox, Manso, Penna and Pará (1942) it was observed that while only 3 lots of vaccine were frankly icterogenic, there were other lots that seemed to possess a definite, though lower, degree of icterogenesis. It would seem most likely that identical factors were operative in Brazil and here; in fact, the workers in Brazil actually suggested the possibility of a moderate multiplication of the icterogenic agent along with yellow fever virus in chick embryos.

J. Chick embryo as a possible source of the icterogenic agent

In the preparation of yellow fever vaccine on a large scale, fertile eggs have been frequently used at the rate of about 10,000 per week. White Leghorn eggs have been used entirely. They were supplied on a contractual basis by Mr. Vincent Darago, who operates a poultry farm of his own and also serves as the manager of the Poultry Farm of the New Jersey Agricultural Experimental Station. The eggs were supplied from two different farms, Mr. Darago's own Shamrock Farm and another owned by Mr. C. R. Parker in Plainsboro, N. J.

An investigation of both farms gave assurance that the poultry in both was healthy. It was stated that if any unusual illness had occurred in the flocks, the egg production would have fallen off. This was not observed. It was stated categorically by Mr. Darago, who has had a great deal of experience in this field, that diseases commonly affecting fowl had been entirely absent, with the exception of fowl paralysis which is known to be endemic in this region and which was occasionally observed in the flocks.

It might be stated in general that, as far as is known at present, diseases affecting poultry show a remarkable species selectivity. Although some viruses causing disease in man and other mammals are also pathogenic for domestic fowl, the reverse has not yet been demonstrated with the possible exception of psittacosis. Furthermore, there are only two diseases of fowl known that apparently are transmitted from one generation to another through the egg. One of these is the pullorum disease, the causative organism of which is easily detected and therefore need not be considered here. The agent of fowl paralysis has not yet been discovered, but it is generally considered to be a virus.

According to Brandly, Waters and Hall (1942), the disease called fowl paralysis, together with several other allied conditions, is now classified as the avian leukosis complex. The paralytic form of this disease complex was first recognized in the United States along the eastern coast about 1920. During the last 20 years the avian leukosis complex has spread rapidly until few flocks of chickens have escaped its ravages. Its yearly toll in this country is believed to exceed that of any other poultry disease.

One of the most obvious symptoms of the avian leukosis complex is a paralyzed condition of one or more parts of the bird's body which led early workers to call the disease fowl paralysis. Later studies revealed a great variety of closely related disease manifestations. The various types recognized now are (*a*) nerve (neural lymphomatosis), (*b*) eye (ocular lymphomatosis), (*c*) internal organ (visceral lymphomatosis), (*d*) bone (osteopetrosis), and (*e*) blood (leukosis). A bird may be affected by one or all of these types at the same time. Jaundice is often associated with the blood and internal organ types with enormous enlargement of the liver. Livers weighing

more than a pound have been found in affected birds weighing less than 4 pounds.

Brandly, Waters and Hall state that most authorities are of the opinion that the cause of the avian leukosis complex with all its various manifestations is a virus or a virus-like agent. The fact that the various types of this complex have been transmitted from bird to bird by different means strongly suggests that it is of an infectious nature and may spread readily. There is definite epidemiological evidence to indicate that the infection is transmitted through the egg. The incubation period has been found to vary from several days to many months (from the time of exposure until symptoms appear). Available evidence suggests that the causative agent possesses great resistance to various physical and chemical inactivating agents.

Although there is a definite possibility that the agent of the avian leukosis complex may have been present in some of the embryos used for the preparation of yellow fever vaccine, there is no evidence available at present that any type of this group is transmissible to man. There is no instance known where the injection of chick-embryo material alone has resulted in hepatitis in man.

K. Possibility of a relationship between yellow fever virus and the hepatitis following vaccination

Conclusive evidence has already been presented by those who investigated the postvaccination outbreaks of jaundice in England and in Brazil that the virus of yellow fever contained in the vaccine was not responsible for the delayed hepatitis which followed the administration of the vaccine. It had been shown that there was no change in the yellow fever antibody level in a patient's serum as the result of an attack of hepatitis. It has also been shown that persons already immune to yellow fever as a result of an actual attack of the disease, but vaccinated in error with icterogenic vaccine, subsequently developed hepatitis. And conversely, nonimmune persons vaccinated with icterogenic vaccine which had lost its virus activity developed jaundice but no immunity to yellow fever.

Additional evidence was obtained during the present outbreak. On February 27th, 1942, a large number of troops were vaccinated against yellow fever with vaccine lot no. 369 at Camp Polk, La. On April 3rd and 4th, blood specimens were obtained from 135 of them and sent to the Laboratories of the International Health Division for yellow fever protection test. Only two of these specimens failed to neutralize yellow fever virus, indicating that the two donors had failed to become immune. Two months later, i.e., 3 months after vaccination, jaundice broke out among the vaccinated men, and there were at least 11 cases among those who were definitely known to have been immune to yellow fever 2 months previously. The early serum specimens from these men had, unfortunately, been discarded so that comparative antibody titrations could not be carried out on the specimens obtained from the same donors 2 months later. However, paired specimens were studied from 15 other men with postvaccination jaundice. Two specimens of serum taken at intervals varying from 1 to 4 weeks during the attack of jaundice from each of these patients were titrated for yellow fever antibodies. No appreciable fluctuation in the antibody level was noticeable in any of them.

In view of the accumulated evidence, therefore, it may be stated definitely

that the virus of yellow fever is not directly responsible for the delayed hepatitis. Whether or not it sometimes plays an accessory role in a synergistic sense is not known.

L. Serum-free yellow fever vaccine

The problem of omitting human serum from yellow fever vaccine had been under study for a number of years, but difficulties were encountered in that much virus was lost during the process of preparation in the absence of serum. These difficulties could not be overcome until recent improvements in the desiccation equipment and technique were made and the practice of storing pulp at low temperature (−75 C) during preliminary sterility tests was adopted. These problems had not yet been solved when the manufacture of vaccine on a large scale was begun. In 1941 a number of experimental lots were made from which serum was omitted entirely. The stability of yellow fever virus in these vaccines was studied, and the results indicated that the virus survived in the rehydrated and diluted vaccine for at least 3 hours at room temperature without appreciable loss in the titer. These were conditions similar to those that would be encountered in the actual administration of the vaccine in the field. However, actual field experience with the new type of vaccine was still lacking. It is true that the field application on a limited scale of the serum-free vaccine had already begun in Brazil, but vaccination there was suspended early in 1941 because of an outbreak of postvaccination encephalitis, and very little could be learned from the experience.

Because of the lack of field experience a general change to the serum-free vaccine at the time was considered unwise. Requests for vaccine were very heavy, and it was considered too risky to introduce a new and as yet untried procedure for general use without an adequate preliminary field trial. The aim was to supply vaccine that was safe and efficient, and all information available from the armed forces indicated that the current vaccine prepared with serum had amply fulfilled these requirements. Furthermore, reports were received from East Africa (A. F. Mahaffy, unpublished communication) indicating that in the administration of an entirely satisfactory vaccine a personal element may play considerable part in the success or failure of vaccination under field conditions. Serum-containing vaccine shipped from New York was used for large-scale vaccination of civilians along the sea coast of Kenya. Subsequent protection tests with sera from vaccinated persons indicated that the vaccine in some localities had failed to immunize from 20 to 25 per cent of vaccinated individuals, while in other places, handled and administered by different officers, the same vaccine produced 100 per cent immunity. The only explanation for such an occurrence seemed to be that in the hands of some medical officers much of the virus in the vaccine becomes inactivated during the rehydration and administration. It might be added that, prior to the present wartime emergency, yellow fever vaccination in South America and elsewhere has always been carried out by physicians who had special training in handling the vaccine. The present large-scale vaccination of the armed forces and civilians in Africa is the first time that the vaccine has been administered entirely by regular medical officers without special training for this purpose.

Within recent months much additional information has become available regarding the characteristics of serum-free yellow fever vaccine. It has been found

that in certain concentration chick-embryo juice is a remarkably efficient protective colloid for the 17D yellow fever virus. Virus suspended in a diluent containing only 3 per cent of chick-embryo juice has remained fully active for 24 hours at room temperature. This is the concentration of embryo juice in the present type of vaccine after it is rehydrated and diluted for human inoculation. During transportation under wartime conditions desiccated vaccine on occasion has been exposed to tropical temperature for several days without appreciable loss in virus titer. The vaccine now contains more chick-embryo protein than there was in the old type serum vaccine, and its virus titer is also higher. One immunizing dose of the present type of vaccine contains about 0.015 ml of chick-embryo juice and considerably in excess of 10,000 mouse lethal doses of 17D yellow fever virus. This is at least 10 times more virus than necessary, but a satisfactory method has not yet been worked out to reduce the virus titer without loss of virus-stabilizing properties of the vaccine.

Very little information is available regarding the efficacy of the vaccine as an immunizing agent when applied under field conditions. Distribution of the serum-free vaccine was begun in June, 1942, and to the time of writing (end of February, 1943) over 6 million doses have been distributed. It is not known how much of it has already been used. To date, only 52 serum specimens taken 1 month after vaccination have been received for yellow fever protection test. All but two were positive, indicating that 96 per cent of the donors had been successfully immunized.

As an additional safety precaution, the size of vaccine lots, which previously amounted to about 80,000 doses each, has now been reduced to between 30,000 and 40,000 doses. Only one-half of a lot is now distributed at a time, the balance being held in storage for a period of 3 months. Should a lot prove through experience to be icterogenic, it is hoped that the icterogenic property would be discovered before the balance of the vaccine was issued, thus limiting the amount of damage to one-half what it would have been otherwise. However, in the absence of a specific test by which the icterogenic property of the vaccine can be detected before the vaccine is distributed for human use, only time will tell whether the measures taken to assure the safety of the vaccine are entirely adequate.

According to Hargett, Burruss and Donovan (1943), the United States Public Health Service began to release serum-free yellow fever vaccine, prepared at the Rocky Mountain Spotted Fever Laboratory, Hamilton, Mont., in the first half of 1942, and since then some 600,000 doses have been released for general use. The vaccine is prepared in the same manner as at the Laboratories of the International Health Division in New York, and the same substrain of the 17D yellow fever virus is used. No unfavorable reactions following the use of this vaccine have been reported. In the same report Hargett and his co-workers also published a set of standard control test requirements to which the vaccine must conform before it is released for human use.

Conclusion

It has not yet been possible to demonstrate conclusively which of the various materials used in the preparation of yellow fever vaccine actually contained the agent responsible for the outbreak of postvaccination jaundice. However, in view of the findings of other investi-

gators that outbreaks of hepatitis have followed the injection of human serum alone, and also that it has been possible to transmit a very similar disease by direct blood or serum transfer from patients suffering from infective hepatitis to healthy volunteers, human serum falls under suspicion much more definitely than any other substance in the vaccine. This suspicion is greatly strengthened by the fact that all highly icterogenic lots of vaccine contained serum from donors who had previously suffered from an attack of a disease commonly called catarrhal jaundice. Furthermore, the evidence presented suggests that a fairly large proportion of normal persons may act as carriers of the icterogenic agent. It is assumed that this agent is probably a virus and that an attack of the disease is probably followed by a carrier state.

Despite the foregoing conclusions, the possibility that the particular variety of yellow fever virus used in the preparation of vaccine may be contaminated with the icterogenic agent derived from human serum cannot be entirely excluded. This virus has had a very long association with human serum, and if the icterogenic agent is capable of multiplication under conditions suitable for yellow fever virus, the possibility of such contamination must be admitted. However, it is felt that if the icterogenic agent should multiply at all under the conditions in which yellow fever vaccine is made, the rate of its multiplication would probably be very much slower than that of yellow fever virus, and therefore high concentration of the agent in the vaccine could probably not be expected. The vaccine at present is made without human serum, and no doubt future experience will reveal whether omission of serum also eliminated the cause of icterus. The peculiar time lag observed in Brazil and here before jaundice appeared makes it exceedingly difficult to estimate just how much experience with a given procedure should be considered sufficient before such a procedure may be judged safe. Two years' observation on 1,300,000 vaccinations in Brazil obviously was inadequate, and the experience in the United States with a considerably larger number during 15 months was also insufficient. Because the so-called catarrhal jaundice, or infective hepatitis, is a relatively common disease, and because at present there is no way by which this condition can be differentiated from the postvaccination hepatitis, it is doubtful whether accurate information can ever be obtained regarding the incidence of jaundice that has followed the use of vaccine lots with a low degree of icterogenesis.

There is no evidence to suggest that the causative agent of postvaccination hepatitis might have originated from the chick embryo. Nor is there evidence to indicate that the yellow fever virus was in any way responsible for the icterogenic property of the vaccine.

III. LABORATORY SEARCH FOR THE ETIOLOGICAL AGENT

In the earlier outbreaks of postvaccination jaundice in England and Brazil much work had already been done in an effort to secure information regarding the etiological agent of the hepatitis. Beyond establishing the fact that the agent was not yellow fever virus, the results had been entirely negative. All attempts to transmit the disease to experimental animals had failed. During the present outbreak similar studies were conducted more or less independ-

ently in 3 different laboratories. This unavoidably involved a certain amount of duplication of effort. In reporting the results from the 3 laboratories on the same general subject, a certain amount of repetition is likewise unavoidable. However, as will be seen below, the main lines of endeavor were different in the 3 laboratories, and therefore the repetition in reporting these studies will deal only with secondary or accessory topics.

A. Laboratories of the International Health Division, New York

The investigations at the Laboratories of the International Health Division in New York were conducted by Dr. Kenneth Goodner, Dr. Max Theiler, and Maj. J. H. S. Gear.[7] The chief subjects investigated included: (1) comparative physicochemical studies on icterogenic and nonicterogenic vaccines; (2) serological studies, especially with a view to developing a specific diagnostic test by which the presence of the icterogenic agent could be detected; (3) attempts to transmit the disease to experimental animals. In addition, as already reported in part II, the relationship of yellow fever virus contained in the vaccine to the postvaccination jaundice was also investigated.

1. *Spectrographic studies of icterogenic and nonicterogenic vaccines*

Inasmuch as there appeared to be a certain similarity between the symptoms of postvaccination hepatitis and those observed in cases of poisoning with such heavy metals as selenium or vanadium, a spectrographic analysis was carried out on a number of the vaccine lots. These included 5 lots that were considered nonicterogenic, 4 lots that apparently possessed a high degree of icterogenicity, and one lot that was moderately icterogenic. Dried vaccine was introduced into bored carbon electrodes that had been thoroughly purified previously. The vaccine-containing electrodes served as anodes while being arced with a 115-volt direct current. The photographs were made with a Hilger quartz spectrograph.

A careful study of the spectrographic plates revealed no features that would seem characteristic of the icterogenic lots of vaccine, and it was entirely impossible to separate the plates into distinct categories in accordance with their absorption spectra. The embryonic material in the vaccines was surprisingly rich in metallic constituents, but these appeared uniformly distributed in both the icterogenic and nonicterogenic lots.

2. *Serological studies*

These consisted largely of precipitation tests in which a serum specimen obtained early from patients suffering from hepatitis was used as an antigen, and a specimen taken late in the disease served as the antiserum. It was hoped that early in the disease there might be present in the circulating blood a specific antigen which in the course of the illness would develop an antibody reacting specifically with the antigen, thus making it possible to evolve an in vitro diagnostic test for the disease.

The results of the study indicated that during the attack of postvaccination hepatitis there did appear a demonstrable antigen-antibody system in the patient's blood. In a number of instances it was shown that serum taken from a patient during the first week of illness gave a

[7] On staff of the South African Institute for Medical Research, temporarily working at the Laboratories of the International Health Division in New York.

positive precipitation reaction when tested against the serum taken from the same patients several weeks later. The presence of such an antigen secured 2 to 7 days after the onset of symptoms was demonstrated in the serum of 7 patients, and precipitating antibodies were demonstrated in the sera of a considerably larger number of patients. On the whole, these reactions were relatively weak and rather difficult to demonstrate. Furthermore, it was soon learned that they were not specific for the disease but appeared to be similar to those described by Hughes (1933) for yellow fever. In fact, the antigen in early specimens of human serum reacted readily to Hughes' antiserum derived from monkeys, and, conversely, the antibody in the serum of patients suffering from postvaccination jaundice gave a strong positive reaction with the Hughes antigen in monkey serum.

Using the Hughes antigen and antibody prepared in monkeys, 74 sera from persons with postvaccination jaundice were tested. Of these, 16 were shown to contain precipitinogen and 47 precipitin. In addition, two sera from cases of jaundice following an attack of dengue fever in persons who had not been vaccinated against yellow fever, also gave positive reactions with the Hughes antigen originating from monkeys. Further studies revealed that the antigen-antibody system, originally observed by Hughes in monkeys infected with yellow fever and subsequently demonstrated in postvaccination hepatitis, is derived from the liver, and that it is organ specific, regardless of the animal species from which the liver originates. Thus the antibody, whether in serum of patients convalescing from an attack of postvaccination jaundice, or in the serum of monkeys recovering from an attack of yellow fever, was found to give positive precipitation reaction with extracts of liver from normal monkeys, guinea pigs, hamsters, cotton rats, white rats and chickens. As was already shown by Hughes, this system is not immunologically related to yellow fever virus, and the reaction cannot be used for diagnostic purposes either in yellow fever or in postvaccination hepatitis.

3. *Attempts to transmit postvaccination hepatitis to laboratory animals*

Much work was done in an attempt to transmit the postvaccination hepatitis to various laboratory animals. These included rhesus monkeys, rabbits, mice in embryonic stage and newborn, cotton rats, Syrian hamsters, chick embryos and very young chicks. Material used for inoculation of these animals consisted of icterogenic vaccine and serum taken from patients early in their attack of postvaccination hepatitis. Various methods of inoculation were tried. The results were entirely negative, and no evidence was obtained that human hepatitis is transmissible to any of the more common laboratory animals.

B. Research Laboratory of the California Department of Public Health

These laboratory investigations of acute hepatitis were conducted by Dr. Eaton in collaboration with Miss Beck and Dr. William D. Murphy. They have been concerned principally with attempts to transmit an infectious agent from jaundice patients to experimental animals and to detect a similar agent in icterogenic lots of yellow fever vaccine. Up to the present the results of these experiments have been inconclusive or negative. Although some suggestive observations have been made, these must be considered preliminary, and for this

reason a description of the experiments at this time will be useful mainly to indicate possible lines of investigation. Serological studies have confirmed and extended experiments originally done in the New York Laboratories of the International Health Division in demonstrating, in the serum from some jaundice patients, substances which gave reactions resembling those of antigen and antibody.

1. *Experimental procedures*

From approximately 30 jaundice patients, blood, duodenal contents and bile, urine, feces and throat washings were collected 1 to 10 days after the onset of symptoms. Liver, spleen, kidney and mesenteric lymph nodes were obtained at autopsy from 5 fatal cases 2 to 6 weeks after onset.[8] From a number of jaundice patients blood specimens were collected at various stages of convalescence for possible use in serological studies. Five icterogenic lots of lyophilized yellow fever vaccine, no. 331, 334, 335, 338 and 367, were used for animal inoculation. Nonicterogenic lots no. 359, 372, 1096 and 1114 were used in control experiments.

Guinea pigs, mice, hamsters, white rats, cotton rats, field mice, voles, ferrets, dogs, cats, chickens, horses and pigs were used as experimental animals. Most animals were inoculated subcutaneously or intraperitoneally with human material or icterogenic vaccines. A few were given blood or autopsy material intracerebrally, and some animals received throat washings or duodenal contents intranasally or orally. The experiments on horses and pigs were conducted by Mr. B. N. Carle and Mr. W. H. Dewhirst, members of the staff of the Division of Veterinary Science at the University of California.[9]

At first the smaller animals were kept under observation for periods of 1 to 2 months. Later the period of observation was extended to 3 months or more. Temperatures were taken daily for a period of 6 weeks on guinea pigs, ferrets, dogs and cats, and for 3 months on horses and pigs. Measurements of icteric index, bilirubin by the quantitative Van den Bergh test, blood counts and other studies of blood chemistry were done on the horses and pigs.

Serial passages of blood, liver, spleen or other tissues of experimental animals were not made except where the results of the primary inoculation were suggestive, as indicated by rise in temperature, increases in the icteric index, or the finding of changes in the livers of animals sacrificed. When such passages were made, similar materials from normal animals were used in control passages when possible.

Chick embryos were inoculated with blood, filtered duodenal contents, filtered suspensions of autopsy material or icterogenic yellow fever vaccine. Three routes of inoculation were used, as follows:

	Routes of inoculation		
	Amniotic	Allantoic	Yolk sac
Age of embryo	12–13 days	11–12 days	6–7 days
Amount of inoculum	0.1 ml	0.1 ml	0.5 ml

Periods of incubation after inoculation ranged from 6 days to 14 days, or the time of hatching. Four to 6 serial passages were carried out with tissues and embryonic fluids irrespective of the presence or absence of pathology in

[8] We are indebted to Col. Dart, and Lt. Col. Biskind, of the Letterman General Hospital for furnishing most of the specimens.

[9] The investigative team is indebted to Prof. C. M. Haring for advice and assistance in planning and organizing these studies.

the embryos. Control passages initiated with broth or normal horse serum were also made.

In experiments with chick embryos inoculated with yellow fever vaccine it was obvious that the yellow fever virus multiplied and was carried in serial passage by the methods used. Therefore, attempts were made to eliminate the yellow fever virus without destroying the icterogenic agent by neutralization with immune monkey serum or by heating to 56 C for 30 minutes.

Histological examinations were done on sections of liver from nearly all of the experimental animals and from chick embryos in the later passages of each series. Tissues were routinely fixed in Zenkers-formalin or Bouin's solution and sections stained by hematoxylin-eosin.

All tissues from animals and chick embryos were cultured in broth and on blood agar in order to exclude the presence of bacteria.

2. *Results of animal inoculations*

The results with guinea pigs, mice, white rats, hamsters, dogs, cats, chickens and pigs were uniformly negative. Questionable degenerative changes of the liver parenchyma were seen in 3 or 4 of about 40 cotton rats and in 2 of 20 peromysces inoculated with human material or icterogenic vaccine. These results were not reproducible. Although serial passages were made in some instances, no infectious agent was definitely demonstrated. Because signs of liver damage were occasionally seen in control animals, it seemed rather likely that intercurrent disease was responsible for these findings.

Mice were inoculated intracerebrally with serum from 25 acute cases of jaundice in order to rule out the possibility of circulating yellow fever virus. Despite the fact that the mice were kept under observation for 6 weeks, none developed symptoms significant of encephalitis.

As hepatitis following immunization with living virus and homologous immune serum had been described in horses by Theiler (1918), by Marsh (1937), and by Shahan, Giltner, Davis and Huffman (1939), it was decided to include horses as experimental animals in the present study. In the first experiment, 5 horses were inoculated. Between 3 and 5 months later, 3 horses in the group developed increases in the icteric index and serum bilirubin of 2 to 3 times. One of these horses had received icterogenic vaccine, another autopsy material and the third a pool of acute-phase blood specimens from jaundice cases. Elevation of the icteric index and serum bilirubin levels continued for 2 to 4 weeks. The horse inoculated with human blood was sacrificed at 5 months and the liver showed a few small areas of fibrosis, but no other changes. Additional horses were inoculated with icterogenic and nonicterogenic yellow fever vaccine, with blood and with autopsy material. In this latter group, 2 of 4 horses receiving icterogenic vaccine developed slightly elevated icteric indices and serum bilirubin. These experiments are not yet completed and details will be presented in a later publication.

One ferret injected intraperitoneally with autopsy material from a jaundice patient showed rather marked liver pathology when sacrificed 116 days after inoculation. Microscopic examination of tissue sections revealed definite fatty degeneration at the periphery of the lobules and a moderate amount of mononuclear infiltration of the portal spaces. The liver of this animal was passed to a second ferret, which died in 45 days with liver pathology resembling that of the

first ferret. No significance can be attached to these observations unless confirmed by further experiments which are in progress in ferrets.

3. *Results of experiments with chick embryos*

Serial passages of chick-embryo material from developing eggs inoculated with specimens of blood, duodenal contents or liver from 11 patients with jaundice gave for the most part negative results.

In two series of allantoic passages, one initiated with filtered duodenal contents and one with filtered liver suspension, about 10 to 30 per cent of the embryos showed signs of degenerative changes in the liver. These were most readily detected by microscopic examination of sections. Several chick-embryo livers had small or large areas of necrosis. In certain passages of these same series the mortality of embryos allowed to be incubated until hatching was higher than in the control eggs, but this increased mortality could not be demonstrated consistently. Similar liver changes were seen less frequently in other series, but they were also found occasionally in the control passages or in normal chick embryos. It is impossible, therefore, to attach definite significance to these findings.

When yellow fever vaccine was inoculated into the yolk sac of chick embryos, the virus multiplied and killed the embryo within 5 to 6 days. When the allantoic sac was inoculated, multiplication of the virus was irregular and death of the embryos usually did not occur. Occasionally, however, yellow fever virus could be demonstrated in chickens even after hatching by subinoculation of the liver and brain into mice. Attempts to eliminate the yellow fever virus by neutralization with immune monkey serum were only partially successful. Although the mixtures of vaccine and immune serum failed to infect mice, multiplication of yellow fever virus occurred after inoculation into the embryos. In about 25 per cent of the eggs, passage of the embryonic tissues to mice by the intracerebral route produced yellow fever encephalitis. In these experiments with chick embryos there was no significant pathology or increase in mortality that could not be attributed to the yellow fever virus itself. The results in embryos inoculated with non-icterogenic lots of vaccine were similar to those obtained with vaccines which had produced jaundice in human beings. Inoculation of vaccine heated to 56 C for 30 minutes caused neither significant pathology nor death in chick embryos. Further passage experiments are now in progress.

4. *Serological studies*

Tests conducted by Maj. Gear at the Laboratories of the International Health Division in New York had revealed that certain acute-phase sera from jaundice patients gave a precipitate when layered over some of the sera taken during convalescence. This phenomenon resembled an antigen-antibody reaction. These observations were continued in California by means of the complement-fixation method.

Sera collected 1 to 15 days after onset of jaundice from 76 patients were tested with a pool of convalescent serum. Of these sera, 19 fixed complement at dilutions of 1 in 4 to 1 in 32 when mixed with the "antibody." Forty-two sera taken between 15 and 150 days after onset gave no reaction with the same antibody. From 27 jaundice patients paired serum specimens were collected during the acute illness and 3 to 5 weeks later. An "antigen" reaction was obtained with

the acute-phase serum specimens of 4 patients, but this reaction disappeared during convalescence.

By use of reactive acute-phase serum as antigen, complement fixation tests for antibody were made with serum specimens from 151 persons with jaundice and 62 persons who had no history of jaundice. In 21 cases of jaundice antibody was found in the serum, but no definitely positive reactions were obtained with sera from normal individuals. No time relation between the occurrence of antibodies and the onset of the disease was demonstrated, positive reactions being obtained with sera taken as early as the second day or as late as the one hundred and fiftieth day after onset. In 5 positive cases from which paired serum specimens were obtained there was no difference between the titer of antibody in the early and late specimens. In preliminary experiments similar reactions have been observed with serum from cases of acute hepatitis or catarrhal jaundice not associated with yellow fever vaccination.

Tests for antibody were also made with suspensions of human liver as antigen. The preliminary results indicate that a higher proportion of positive reactions may be obtained with serum from jaundice patients by this method. Suspension of liver from a patient with a fatal case of coronary occlusion without history of jaundice gave reactions equal to, or greater than, those obtained with similar liver preparations from jaundice patients.

C. Laboratories of The Hooper Foundation, University of California

1. *Attempts to transmit postvaccination hepatitis to monkeys*

These studies were conducted by Dr. Meyer in cooperation with B. Howitt, H. Sommer, L. Foster, and J. Adatto. Material for study was received from patients under observation at the Letterman General Hospital, San Francisco, and at Hoff General Hospital, Santa Barbara. The specimens of blood, duodenal bile or nasal washings, as well as those from livers and spleens taken at autopsy, were inoculated into rhesus monkeys by various routes. Thirty-five monkeys were used, as shown in table 42.

The blood and the duodenal bile specimens sent from Hoff General Hospital were collected from a number of patients. In order to test these specimens on the limited number of monkeys available, pools were made of both types of material. Pool 1 consisted of defibrinated blood from 6, pool 2 from 5, and pool 3 from 5 patients, while pool 1a contained duodenal bile from 3, and pool 2a from 5 patients whose blood was tested. Specimens from all the other patients were not pooled but were inoculated as follows:

Undefibrinated citrated blood was injected intraperitoneally into 5 monkeys.
Blood defibrinated with glass beads was injected intraperitoneally into 5 monkeys.
Defibrinated blood was injected intranasally and intraperitoneally into 5 monkeys.
Defibrinated blood was injected intravenously and intraperitoneally into 3 monkeys.
Duodenal bile was given intraperitoneally alone to 4 monkeys.
Duodenal bile was given intranasally and intraperitoneally to 4 monkeys.
Duodenal bile and nasal washings were given to 3 monkeys.
Duodenal bile was fed by stomach tube to 2 monkeys.

Suspensions prepared in 10 per cent concentrations from the liver and spleen of two patients and the brain from one patient who died of jaundice were inoculated by several different pathways including the intravenous route, as follows:

Liver suspensions were administered intraperitoneally, intravenously and subcutaneously to 3 monkeys.

TABLE 42

Histories of monkeys injected with specimens obtained from jaundice patients

Monkey number	Inoculation: amount (*ml*) and route*	Material	Patient and hospital	Date collected	Date of inoculation	Reinoculation	Icteric index			Temperatures (*centigrade*)			Results
							Maximum	Minimum	Average	Maximum	Minimum	Average	
2649	4 i.p.	Whole blood	Letterman Hospital; [redacted]	3–28–42	3–28–42		0.25	0	0.07	39.9	38.5	39.6	Monkey in good health 4 months later
2651	20 i.p.	Duodenal bile	Letterman Hospital; [redacted]	3–28–42	3–28–42		0.15	0	0.07	39.7	38.8	39.4	Monkey in good health 4 months later
2595	4 i.p.	Whole blood	Letterman Hospital; [redacted]	4–1–42	4–3–42	4–6–42 5 ml i.n. clot	0.25	0	0.16	39.3	38.1	38.6	Monkey in good health 4 months later
2650	20 i.p.	Duodenal bile	Letterman Hospital; [redacted]	4–1–42	4–3–42		0.2	0	0.09	39.2	38.0	38.9	Monkey in good health 4 months later
2654	3 i.p.	Whole blood	Letterman Hospital; [redacted]	4–1–42	4–3–42	4–6–42 5 ml i.n. clot	0.3	0	0.12	39.6	38.5	39.0	Monkey in good health 4 months later
2656	20 i.p.	Duodenal bile	Letterman Hospital; [redacted]	4–1–42	4–3–42		0.25	0	0.09	39.8	37.7	39.1	Monkey in good health 4 months later
2702	4 i.p.	Whole blood	Letterman Hospital; [redacted]	4–1–42	4–3–42	4–6–42 5 ml i.n. clot	0.2	0	0.03	39.8	38.6	39.2	Monkey in good health 4 months later
2716	10 i.p.	Duodenal bile	Letterman Hospital; [redacted]	4–1–42	4–3–42		0.25	0	0.1	40.1	38.9	39.3	Monkey in good health 4 months later
2799	3.5 i.p.	Whole blood	Letterman Hospital; [redacted]	4–1–42	4–3–42	4–6–42 5 ml i.n. clot	0.35	0	0.14	39.9	39.1	39.4	Monkey in good health 4 months later
2717	25 i.p.	Duodenal bile	Letterman Hospital; [redacted]	4–1–42	4–3–42		0.15	0	0.03	40.5	38.8	39.3	Had increased temperature for 3 days about *11 days* after inoculation. Recovered
2860	5 i.n. 20 i.p.	Defibrinated blood	Pool no. 1, Hoff Gen. Hosp.	4–3–42	4–6–42	4–8–42 5 ml i.n. 5 ml i.p.	0.25	0	0.13	40.0	39.0	39.6	Monkey in good health 4 months later
2861	6 i.n. 10 i.p.	Duodenal bile	Pool no. 1a, Hoff Gen. Hosp.	4–3–42	4–8–42		0.2	0.1	0.13	39.6	38.5	39.1	Monkey in good health 4 months later
2862	5 i.n. 20 i.p.	Duodenal bile	Pool no. 2a, Hoff Gen. Hosp.	4–3–42	4–8–42		0.15	0.05	0.075	40.0	38.3	39.1	Monkey in good health 4 months later
2863	5 i.n. 20 i.p.	Defibrinated blood	Pool no. 2, Hoff Gen. Hosp.	4–3–42	4–8–42	4–10–42 6 ml i.n. 5 ml i.p.	0.25	0.1	0.13	40.0	39.0	39.7	Monkey in good health 4 months later

TABLE 42—*Continued*

Monkey number	Inoculation: amount (*ml*) and route*	Material	Patient and hospital	Date collected	Date of inoculation	Reinoculation	Icteric index			Temperatures (*centigrade*)			Results
							Maximum	Minimum	Average	Maximum	Minimum	Average	
2864	5 i.n. 17 i.p.	Defibrinated blood	Pool no. 3, Hoff Gen. Hosp.	4–6–42	4–8–42		0.1	0	0.04	39.5	38.8	39.4	Monkey in good health 4 months later
2865	5 i.n. 6 i.p.	Defibrinated blood	Letterman Hospital; GRO-A	4–10–42	4–10–42	4–14–42 Blood i.n.	0.4	0	0.085	40.6	38.5	38.8	Increased temperature for 4 days, beginning *4 days* after inoculation. Recovered
2868	20 i.n.	Throat washings	Letterman Hospital GRO-A	4–10–42	4–11–42		0.2	0	0.03	40.4	38.6	39.5	Increased temperature for 4 days, beginning *9 days* after inoculation. Recovered
	4 i.n.	Duodenal bile	Letterman Hospital GRO-A	4–10–42	4–12–42								Monkey in good health 4 months later
	5 fed by tube	Duodenal bile	Letterman Hospital GRO-A										Monkey in good health 4 months later
	10 i.n.	Nasal washings	Letterman Hospital; GRO-A	4–10–42	4–13–42								Monkey in good health 4 months later
2866	5 i.n. 7 i.p.	Defibrinated blood	Letterman Hospital; GRO-A	4–10–42	4–10–42	4–14–42 5 ml i.n.	0.2	0	0.037	39.7	38.8	39.3	Monkey in good health 4 months later
2867	18 i.n.	Nasal washings	Letterman Hospital; GRO-A	4–10–42	4–10–42	4–12–42 5 ml i.n. bile	0.3	0	0.1	40.0	39.1	39.3	Monkey in good health 4 months later
	1.75 i.n.	Bile	Letterman Hospital GRO-A	4–10–42	4–10–42	4–14–42 5 ml i.n. bile							Monkey in good health 4 months later
2869	6 i.n. 6 i.p.	Defibrinated blood	Letterman Hospital; GRO-A	4–16–42	4–17–42	4–20–42 2 ml i.n.	0.25	0	0.09	39.7	39.0	39.3	Monkey in good health 4 months later
2870	5 i.n. 6 i.p.	Defibrinated blood	Letterman Hospital; GRO-A	4–16–42	4–17–42	4–20–42 3 ml i.n.	0.3	0	0.13	40.0	39.0	39.6	Monkey in good health 4 months later
	10 fed by tube	Duodenal bile	Letterman Hospital; GRO-A	4–16–42	4–20–42								Monkey in good health 4 months later
2871	5 i.n. 20 i.p.	Duodenal bile	Letterman Hospital GRO-A	4–16–42	4–17–42		0.1	0	0.05	39.7	39.0	39.3	Monkey in good health 4 months later
2873	20 i.n.	Nasal washings	Letterman Hospital; GRO-A	4–17–42	4–17–42	4–20–42 6 ml bile by tube	0.1	0	0.031	39.7	38.8	39.2	Monkey in good health 4 months later
	20 i.p.	Duodenal bile	Letterman Hospital GRO-A										Monkey in good health 4 months later

TABLE 42—*Continued*

Monkey number	Inoculation: amount (*ml*) and route*	Material	Patient and hospital	Date collected	Date of inoculation	Reinoculation	Icteric index			Temperatures (*centigrade*)			Results
							Maximum	Minimum	Average	Maximum	Minimum	Average	
2872	5 i.n. blood 17	Duodenal bile	Letterman Hospital; [redacted]	4–16–42	4–17–42	4–20–42 Blood 20 ml fed	0.15	0	.08		38.0	39.1	Died of dysentery about 3 weeks after the inoculations
2874	5 i.n. 6 i.p.	Defibrinated blood	Letterman Hospital; [redacted]	4–17–42	4–17–42	4–20–42 4 ml blood i.n.	0.1	0	0.042	39.7	39.0	39.2	Monkey in good health 4 months later
2876	2.5 i.c.	Spinal fluid	Letterman Hospital; [redacted]	5–6–42	5–7–42		0.15	0	0.03	39.2	37.9	38.6	Monkey in good health 4 months later
	2 i.v. 4 i.p.	Defibrinated blood	Letterman Hospital; [redacted]	5–7–42	5–8–42								Monkey in good health 4 months later
2888	2 i.v. 5 i.p.	Liver (10%)	Letterman Hospital; [redacted]	5–23–42	5–24–42	5–27–42 3.5 ml i.p.	0.2	0	0.08	39.3	38.4	38.9	Monkey in good health 4 months later
2889	5 i.p.	Spleen (10%)	Letterman Hospital; [redacted]	5–23–42	5–24–42	5–27–42 5 ml i.p.	0.1	0	0.03	39.0	38.0	38.5	Monkey in good health 4 months later
2878	4 i.v. 5 i.p.	Defibrinated blood	Letterman Hospital; [redacted]	5–15–42	5–15–42		0.1	0	0.03	39.9	39.0	39.3	Monkey in good health 4 months later
2879	20 i.p. 25 i.n.	Duodenal bile	Letterman Hospital; [redacted]	5–15–42	5–15–42		0.2	0	0.07	39.5	38.3	38.9	Monkey in good health 4 months later
2882	20 i.p. 5 i.p. 2 i.c.	Liver (10%)	Letterman Hospital; [redacted]	5–15–42	5–16–42	5–18–42 10 ml i.p.	0.15	0	0.06	39.7	38.6	39.3	Monkey in good health 4 months later
2885	2 i.v. 5 i.n. 2 i.c.	Spleen (10%)	Letterman Hospital; [redacted]	5–15–42	5–16–42	5–18–42 5 ml i.p.	0.1	0	0.02	39.5	38.4	39.1	Monkey in good health 4 months later
2886	3 i.v. 5 i.p. 5 i.n. 3 i.c.	Liver (10%)	Letterman Hospital; [redacted]	5–15–42	5–16–42	5–18–42 3 ml i.v. 6 ml i.p.	0.2	0	0.06	39.6	38.6	39.1	Monkey in good health 4 months later
2890	4 i.v. 10 i.p.	Defibrinated blood	Letterman Hospital; [redacted]	6–3–42	6–4–42		0	0	0	40.4	38.2	39.0	Died of dysentery 24 days after the inoculation
2893	3 i.c. 10 i.p. 5 i.n.	Brain (10%)	Hoff Gen. Hosp. [redacted]	7–15–42	7–16–42		0	0	0	39.8	38.0	39.0	Developed a prolapsed rectum; operated upon; recovered and well 4 months later

* i.p. = intraperitoneal; i.c. = intracranial; i.v. = intravenous; i.n. = intranasal.

Spleen suspensions were similarly administered to 2 monkeys.

Brain suspensions were administered intracranially, intraperitoneally and intranasally to one monkey.

Daily temperatures were taken on all animals for at least 1 month after the initial inoculation and observations were made on the general behavior or the appearance of jaundice for 4 months. From 3 to 5 ml of blood were removed from the vein of each animal at least once a week for about 2 months and at any time when the temperature or general condition of the monkey warranted an estimation of the icteric index. Dr. Sommer conducted the tests for bilirubin.

The monkey sera were first compared with the potassium chromate standards, giving the icteric indices directly.

They next were treated according to the indirect Van den Bergh test. In numerous instances the direct Van den Bergh test, as well as variations of the indirect test, was tried by precipitation before or after diazotization. Both ferric sulfocyanate and cobalt sulfate were used as comparator solutions.

The various methods were frequently checked with normal and icteric human sera and found to yield the expected results.

All the monkey sera examined showed between 0 and 0.4 units of bilirubin, values which fall well within the normal range for human sera.

Normal icteric index values were determined on the animals before they were inoculated. Minor fluctuations were encountered in the course of the observations, but on no occasion was real icterus noted. The minimum, maximum and average readings made on the sera of the experimental animals are recorded in table 42.

Total blood counts were made twice a week with occasional white counts 3 times a week for a period of 1 month. During the remainder of the observation period only 1 count was made at weekly intervals. The total number of the red and white cells fluctuated within normal limits (r.b.c. average 5,590,000 and w.b.c. 16,210 per cu. mm.). However, the white cell counts varied considerably (up to 28,800). Some monkeys constantly showed a greater number of polymorphonuclear cells than lymphocytes (73 polymorphonuclear cells to 27 lymphocytes), while others presented an opposite blood picture (72 lymphocytes to 27 polymorphonuclear cells). An increase of monocytes (14 to 51 per cent) was noted in two monkeys (no. 2872 and 2890) during the terminal phases of an intercurrent bacillary dysentery infection.

The temperatures ran a normal course except in 3 animals which showed a febrile rise for 3 to 4 days from 3 to 11 days after the inoculation. Recovery from the temporary febrile state was prompt and complete. None of the monkeys with fevers developed jaundice. One animal with bacillary dysentery had a slight fever; it ultimately succumbed to the infection.

Specimens of liver, spleen and brain taken at autopsy from 3 jaundice patients failed to induce any noticeable symptoms. One animal injected with brain material developed a rectal prolapse, but made an uneventful recovery following operation.

The following conclusions were reached: Attempts to demonstrate an active disease-producing agent in whole or defibrinated blood, duodenal bile and nasal washings collected from 32 patients suffering from jaundice, or in the organ suspensions of liver, spleen and brain from 3 patients with fatal postvaccinal jaundice, injected individually or in pools by various routes into rhesus monkeys (*Macaca mulatta*), have been unsuccess-

ful. All the animals were kept under observation for 4 months, and 15 even for 6 months. Systematic blood studies have failed to indicate a rise in the biliary pigments of the blood serum or a change in the percentage distribution of the blood cells except when the monkeys suffered from bacillary dysentery.

2. *Examination of sera from patients with jaundice for leptospirosis*

Early in March, 1942, the Laboratory of the Ninth Service Command requested the examination of 11 sera for the presence of agglutinins and lysins against *Leptospira icterohaemorrhagiae*. In rapid sequence the Air Base at Fresno, Fort Douglas and other military establishments submitted samples for serologic tests of icteric sera obtained from patients who were hospitalized on account of jaundice suspected of being in some way related to the yellow fever vaccinations. A modified Schüffner technique in use at the George Williams Hooper Foundation since 1936 was employed. The procedure is generally known as the porcelain-plate method (Meyer et al., 1939). Both live and formalin-killed antigens of *Leptospira icterohaemorrha-*

TABLE 43

Leptospira-agglutination test with sera from soldiers and civilians

	Origin of sera	Total sera	Agglutination reaction with *L. icterohaemorrhagiae*				Agglutination reaction with *L. canicola*			
			1 in 30	1 in 100	1 in 300	1 in 3,000	1 in 30	1 in 100	1 in 300	1 in 3,000
12 army establishments (54 specimens)	Fort Douglas	14	0	0	0	0	0	0	0	0
	Air Base, Fresno	12	0	0	0	0	0	0	0	0
	Ninth Service Command Lab-	11	0	0	0	0	0	0	0	0
	Camp Roberts [oratory	4	0	0	0	0	0	0	0	0
	Hoff General Hospital	4	0	0	0	0	0	0	0	0
	Camp Cooke	2	0	0	0	0	0	0	0	0
	Camp Haan	2	0	0	0	0	0	0	0	0
	Camp Callan, Fort Ord, Hamilton Field, 73D Evacuation Hospital, Letterman Hospital. One specimen each	5	0	0	0	0	0	0	0	0
8 cities in California (34 specimens)	San Francisco	24	3	1	1	1	3	0	0	0
	Berkeley	4	0	0	0	0	0	0	0	0
	Los Angeles, Long Beach, Gonzales, Oakland, Monterey, Richmond. One specimen each	6	0	0	0	0	0	0	0	0
6 cities in other parts of the United States and in Canada (61 specimens)	Detroit, Michigan	48	21	4	1	1	10	0	0	0
	Iowa	1	1	0	0	0	0	0	0	0
	Canada	4	1	1	0	0	1	0	0	0
	Ohio	4	1	0	0	0	0	0	0	0
	Arizona	2	0	0	0	0	0	0	0	0
	Oregon	1	0	0	0	0	0	0	0	0
	Nebraska	1	0	0	0	0	0	0	0	0
Totals 149		149	27	6	2	2	14	0	0	0

giae (San Francisco and Boston strains), *L. canicola*, and occasionally *L. hebdomadis*, *L. bellico*, *L. salinem*, and *L. rachmat* have been incorporated in the test series. During the period in which the army hospitals submitted sera, the laboratory conducted serologic tests with blood specimens collected from civilians living in widely separated sections of California, and several cities in other parts of the United States and in Canada. The results of these tests are summarized in table 43.

It is noteworthy that not one of the 54 serum specimens, obtained from cases clinically and epidemiologically diagnosed as postvaccination jaundice, agglutinated or lysed *Leptospira icterohaemorrhagiae* or *L. canicola* even in the lowest dilutions. In January a typical case of leptospirosis or Weil's disease was diagnosed in San Francisco; the serum agglutinated *L. icterohaemorrhagiae* in a dilution of 1 in 3,000. During February and March the sera of 3 patients gave nonspecific reactions in a dilution of 1 in 30, while the remaining 20 sera from San Francisco and 10 others from civilians throughout California revealed no agglutinins. With the exception of Detroit, where clinical and subclinical leptospirosis had previously been proved, no evidence of widespread leptospirosis was secured. In fact, preliminary epidemiologic investigations of several army posts where jaundice had appeared were proved to be exceptionally free from rodents or stagnant water pools which might have served as sources for leptospiral infections.

IV. FINDINGS AND RECOMMENDATIONS

A. Findings

1. The greater part of the jaundice which was epidemic in army personnel in the western region of the United States from March to July, 1942, was a hepatitis similar in its manifestations to the common so-called catarrhal jaundice, or infective hepatitis, but related to the injection of certain lots of yellow fever vaccine from 60 to 150 days before the attacks.

2. In the western region, as defined in this report, the total number of reported cases of jaundice of the type under study was 11,853, and the records of 10,284 of these were subjected to statistical analysis. In this latter group there were 31 fatal cases, giving a mortality rate of 3 per 1,000 cases.

3. There were few, if any, secondary cases and no evidence was obtained that the disease was derived from the civilian population or transmitted to it, but, as would be expected, there were scattered cases of jaundice unrelated to vaccination in soldiers and also in civilians near camps. In Hawaii, according to the statistical analysis, these unrelated cases must have been fairly numerous, but in most other places they seem to have been few.

4. The absence of any noticeable increase in jaundice in the Navy and in foreign countries where yellow fever vaccine from the same source was used on a large scale was found to be due to the fact that by unusual chance they did not receive the definitely icterogenic lots. That the immunity of the Navy could thus be explained was confirmed by the presence of a considerable proportion of cases of jaundice in a small number of persons who had received vaccine from one of the icterogenic lots supplied in quantity to the Army.

5. The nature of the icterogenic agent in the vaccine could not be definitely

determined, but several possibilities were ruled out. The jaundice was not due to the modified yellow fever virus in the vaccine, to insanitary conditions, to food or water, to contact with any chemical poison, to inoculation to prevent diseases other than yellow fever or to any local environmental factor.

6. The most plausible hypothesis as to the source and nature of the icterogenic agent was that it had been introduced into the vaccine in human blood serum secured from supposedly normal donors and used in manufacture, and that the agent was most probably an unknown filterable virus capable of causing disease in man and occasionally circulating subsequently in his blood during a prolonged carrier state. This hypothesis was supported by the published reports of similar cases and outbreaks of jaundice after inoculation with serum-containing yellow fever vaccine or pappataci fever vaccine, and after injections of measles or mumps convalescent serum, or blood plasma or serum. Thorough inquiry into the methods of vaccine manufacture and materials used failed to reveal any other plausible explanation.

7. Numerous attempts to isolate the hypothetical virus through inoculation of various experimental animals with incriminated vaccine or with blood, tissues or excreta from jaundiced patients gave either completely negative or only suggestive results. Leptospiral jaundice was ruled out.

B. Recommendations

1. On April 13th, 1942, the investigative team telephoned the following preliminary recommendations to the Surgeon General of the Army:

"That, as far as military exigencies permit, vaccination with lots with numbers over 330 be suspended for at least two months for the purpose of permitting the completion of the investigations in progress and allowing any delayed manifestations to appear and indicate whether later lots are safe. In the meanwhile it is suggested that troops destined for countries of exceptional exposure be immunized as far as possible with vaccine obtained from the laboratory of the United States Public Health Service in Hamilton, Montana, or from the laboratory in Bogotá, Colombia, in which places the eggs and serum used in manufacture are obtained from sources different from those around New York."

The action taken by the Surgeon General of the Army after the receipt of the these recommendations was to stop the use of all yellow fever vaccine manufactured by the International Health Division in New York for the time being and to use vaccine of the same type prepared by the United States Public Health Service in the Rocky Mountain Laboratory of the National Institute of Health at Hamilton, Mont. It was not found necessary to call on the Yellow Fever Laboratory in Bogotá, Colombia, for additional vaccine. The arrangement was to be in effect until the problems presented by the jaundice epidemic had been satisfactorily settled. It was decided also to limit the vaccination of army personnel to persons traveling to, or through, or stationed in areas in which yellow fever is endemic, and such areas were defined.

2. The investigative team now recommends that only serum-free yellow fever vaccine be utilized in the immunization of military personnel against yellow fever. During the course of the investigation the two laboratories preparing the vaccine in the United States found ways to modify their methods so that human serum could be omitted.

3. The problem of jaundice following vaccination against yellow fever may have been completely solved by the exclusion of human serum from the vaccine, and the lapse of more time with continued absence of jaundice from large numbers of vaccinated persons will add to the assurance that the danger is past. The risk, however, may be more difficult to eliminate in the case of other serum-containing biologics, blood plasma and serum needed by the military forces and civilians as well. The team therefore recommends that all possible encouragement and assistance be given to research aimed at isolating the causative agent of postinoculation jaundice, devising methods for its detection, and finding ways of excluding it from serum-containing biologic products.

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