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JAUNDICE FOLLOWING ADMINISTRATION OF HUMAN SERUM

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JAUNDICE following administration of materials containing serum of the homologous species has been observed repeatedly both in man and in lower animals. It is still unknown whether this type of jaundice is identical with naturally occurring socalled infectious hepatitis.

One of the earliest reports of this condition is that of Hirsch[1] who records an outbreak in 1883-4 of jaundice among individuals vaccinated with "humanized lymph in glycerine." Among 1,289 persons vaccinated, 191 or 14.8 per cent developed jaundice after incubation periods "extended to several weeks and even to a couple of months." No cases developed among 500 person vaccinated with a different lymph.

In 1918 Theiler[2] reported a condition known as "staggers" in horses, which followed administration of homologous serum. Jaundice was a marked sign. The incubation period varied from 27 to 165 days and the mortality varied from 4 to 18 per cent among large groups of immunized animals. Slagsvold[3] found that 101, or 4.2 per cent of 2,400 horses treated with anti-anthrax serum developed liver damage from 8 to 95

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days following injection of the material. In most cases the elapsed period was 50 to 60 days. A similar condition in horses following the administration of equine encephalomyelitis vaccine containing homologous serum has been observed in this country.[4, 5]

MacNalty[6] drew attention to the occurrence of jaundice among 37 of 82 to 109 persons who had been given convalescent measles serum from the same pool of material. Seven deaths were recorded. Convalescent measles serum in doses of 4.5 cc. was given to seven children and in 75 to 83 days they all developed severe jaundice. Three cases terminated fatally. Two months later two children who had had contact with these patients developed mild infective hepatitis.[7]

Findlay and MacCallum[8] found that hepatitis was a complicating factor in the use of yellow fever vaccines. Different types of vaccines used produced jaundice; the only common components in the products were human serum and the attenuated virus of yellow fever. During a five-year period 3,100 persons were immunized against yellow fever and 89, or 2.87 per cent, of these developed jaundice. The average incubation period was 2 to 3 months with a range of 36 days to 7 months. Hepatitis has been associated with the use of yellow fever vaccine by other workers. Soper and Smith[9] described the first series of cases occurring in South America. A vaccine containing immune monkey serum and tissue culture virus was used. Among 244 persons immunized, 66, or 27 per cent, developed hepatitis. The incubation period was prolonged.

Fox and his coworkers[10] studied two outbreaks of hepatitis following the use of yellow fever vaccine in Brazil. In 1939, 304 persons were immunized and 27 per cent suffered from hepatitis during the fourth and fifth months following immunization. In 1940, 35 lots of vaccine were used to vaccinate 107,169 persons. There were only 93 cases, or 0.1 per cent, of jaundice among 87,878 persons given material from 33 lots of vaccine. Two other lots produced a greater amount of jaundice among the recipients. The attack rate with one lot used among 9,604 individuals was 7.68 per cent and for another lot given to 9,587 persons the rate was 1.56 per cent. The average incubation period was 24.8 weeks for the 33 lots of vaccine, 17.8 weeks for the lot producing the highest attack rate of icterus, and 20.4 weeks for the other lot. A total of 25 deaths was recorded.

Recent U. S. Army experience with yellow fever vaccine resulted

in 28,585 cases of hepatitis with 62 deaths as of July 24, 1942.[11]

Immunization against pappataci fever has also resulted in the appearance of jaundice after a prolonged period. The method used for immunization consisted of separate inoculations of virus and antiserum. The source of virus was human blood obtained during the acute stage of the disease. Sergiev *et al*,[12] studied 100 cases of jaundice resulting from the use of such materials. The incubation period varied from 63 to 146 days; about 50 per cent of the cases occurred between 85 and 95 days after inoculation. About 30 per cent of those vaccinated subsequently developed hepatitis.

A report of the British Ministry of Health[13] attributes 48 cases of jaundice with 8 deaths to the use of pooled convalescent and adult human serum for measles. Incubation periods ranged from 16 to 161 days. Twelve cases of jaundice due to transfusion were also recorded. A number of other cases of hepatitis developed following the use of mumps convalescent serum. The writers conclude that, "any doubt as to the reality of the association is removed by the frequency with which hepatitis has followed the injection of human blood products."

In addition to the reports of jaundice secondary to immune sera and supposedly normal serum, there were in 1943 two reports of jaundice following the administration of pooled human plasma, reconstituted dried human serum and blood transfusion. Beeson[14] in this country reported seven cases. Of these, four had received one or more transfusions of citrated blood only. The other three persons had received both pooled plasma and citrated blood at different times. In five of the cases the minimum possible incubation period varied from 69 to 111 days. In England, Morgan and Williamson[15] saw nine cases which developed following transfusion with serum, plasma or both. The incubation periods varied from 7 to 16 weeks.

Findlay and Martin[16] present evidence that an "infective icterogenic agent" is present in the nasopharynx of individuals developing hepatitis following administration of yellow fever vaccine. While some doubt might be maintained concerning two of the cases produced by this experimental approach, the evidence in the third case (Case 2) seems convincing. Voegt[17] attempted to transmit the agent of infective hepatitis from one person to another with results which are suggestive.

Lainer[18] carried out a human inoculation experiment in which he inoculated healthy persons with blood or duodenal juice from patients

three to ten days after the appearance of jaundice. The duodenal juice was introduced into the duodenum by tube and blood was given by transfusion. The study was stated to be entirely negative; duration of observation was not given.

The relation between jaundice following administration of homologous serum and the disease known as infectious hepatitis or epidemic catarrhal jaundice is not known. According to the work of Cullinan,[19] Pickles,[20] and others, infectious hepatitis is spread by droplet infection and has an incubation period varying from 20 to 40 days. Two epidemics, one in Canada, the other in Sweden, reported by Fraser[21] and Hallgren[22] appear to have been due to contamination of drinking water by sewage; transmission by the respiratory tract was not seen. Very few secondary cases have been attributed to association with cases of jaundice following the administration of homologous serum. This disease is further peculiar in that the incubation period is prolonged considerably over that of infectious hepatitis.

Many attempts to isolate the causative agent of infectious hepatitis and serum-induced jaundice have been made, so far without success. It is generally supposed that it is a virus.

Isolation of an etiologic virus was claimed by Siede and Meding[23] who inoculated the chorioallantois of chick embryos with duodenal juice obtained from a jaundiced patient. Proof of isolation consisted only in the ability of their material to kill the chick embryos in 4-5 days. The lethal agent was lost on the fourth transfer.

An opportunity to study an epidemic of jaundice following the use of yellow fever vaccine presented itself when an outbreak occurred in the Virgin Islands in the summer of 1942. Sufficient epidemiological evidence[24] was accumulated to establish the identity of the disease and material was obtained for study. Since August 1942 a human inoculation study has been carried on in an effort to gain further information concerning the nature and etiology of this condition.

Epidemiology

During 1942 a total of 11,358 individuals on the islands of St. Thomas and St. John, Virgin Islands, was inoculated with lot 331 yellow fever vaccine containing pooled human serum. According to reliable data there were 11,265 persons living on the island of St. Thomas and 765 on the island of St. John, but owing to wartime increases in

TABLE I

DATES OF IMMUNIZATION AND NUMBERS OF PERSONS VACCINATED WITH YELLOW FEVER VACCINE IN THE VIRGIN ISLANDS

Date immunized, 1942	*Place*	*Number immunized*	*Date immunized, 1942*	*Place*	*Number immunized*
Mar. 4	St. Thomas	490	Mar. 16	St. Thomas	1,554
Mar. 5	do	403	Mar. 18	do	1,505
Mar. 6	do	624	Mar. 20	do	2,568
Mar. 9	do	1,134	Mar. 23	do	298
Mar. 10	do	392	Mar. 28	do	410
Mar. 11	do	596	Apr. 17	St. John	211
Mar. 12	do	597			
Mar. 13	do	576	Total		11,358

population these figures may be too low. There were 11,147 persons vaccinated with yellow fever vaccine on St. Thomas between March 4 and March 28, 1942, and 211 persons vaccinated on St. John on April 17, 1942. The same lot of vaccine was used throughout the immunization procedure. The dates of vaccination together with the numbers of persons vaccinated are given in Table I.

Jaundice was first noted in May, and by June 2, 1942, about 50 cases had been observed. During the next two weeks it was estimated that between 300 and 500 cases occurred. In order to obtain some exact knowledge concerning the incidence of the disease following the administration of vaccine, a survey was done in the city of Charlotte Amalie, St. Thomas, between July 6 and July 16. A group of 1,198 persons was studied. This sample is roughly 10 per cent of the population involved. The data obtained from this survey are included in Table II. It was established that 14.7 per cent of the vaccinated individuals developed symptoms of hepatitis following vaccination and that the incidence was greatest in the age groups between 20 and 59 years. Among 159 persons who were said not to have been vaccinated, 3 cases occurred. Inasmuch as the vaccination records were not entirely adequate it is somewhat difficult to assess the significance of these cases.

Conditions were not suitable for accurate determination of the incubation period. Among a group of 75 patients from whom reliable and

TABLE II

SAMPLE SURVEY OF CHARLOTTE AMALIE, VIRGIN ISLANDS, SHOWING INCIDENCE OF JAUNDICE FOLLOWING YELLOW FEVER IMMUNIZATION

Age	*Vaccinated population*			*Unvaccinated Population*			*Total*		
	Number Surveyed	*Number of cases of hepatitis*	*Per cent with hepatitis*	*Number Surveyed*	*Number of cases of hepatitis*	*Per cent with hepatitis*	*Number Surveyed*	*Number of cases of hepatitis*	*Per cent with hepatitis*
Under 1	31	0	7.5	17	....	....	297	20	6.7
1—4	127	11		13	....				
5—9	107	9		2	....				
10—14	120	16	11.8	3	....	....	260	29	11.3
15—19	125	13		12	....				
20—24	119	19	18.8	16	....	....	236	40	16.9
25—29	88	20		13	1				
30—34	76	20	21.1	11	....	....	153	28	18.3
35—39	57	8		9	....				
40—44	45	8	22.8	6	....	....	77	16	20.8
45—49	25	8		1	....				
50—54	48	9	21.4	4	....	....	85	15	17.7
55—59	22	6		11	....				
60—64	18	2	12.2	13	1†	....	90	8	8.9
65—69	19	2		10	....				
70+	12	2		18	1				
	1,039	153*	14.7	159	3	1.9	1,198	156	13.0

* Including 12 cases with all signs and symptoms except icterus.

† 1 case with all signs and symptoms except icterus.

observed data could be elicited the average period between immunization and development of hepatitis was 103 days with a range of 75 to 130 days.

The disease was similar to that noted by other observers. Clinically, the disease varied considerably from very mild to extremely severe cases. Onset usually began with headache, pains in the shoulders and back, and frequently with pains in the fingers. A sensation of fullness in the epigastrium was quite characteristic and with this was associated anorexia and nausea. Weakness was a common complaint. Within a day or so the urine was noted to be very dark and within 2 to 3 days icterus of the sclerae appeared. Constipation and clay-colored stools were noted

TABLE III

DATA CONCERNING VIRGIN ISLANDS SERUMS USED IN POOL FOR EXPERIMENTAL GROUPS 2 AND 6

Number	*Date vaccinated, 1942*	*Date jaundiced, 1942*	*Date bled, 1942*	*Results of quantitative van den Bergh test*	*Remarks*
				(*mg. per 100 cc.*)	
6	Mar. 4	June 22	July 6	0.68	.....
11	Mar. 16	June 28	do	1.07	.....
30	Mar. 4	....	July 8	0.62	.....
40	Mar. 20	....	do	0.56	.....
43	Mar. 6	....	do	1.16	.....
47	Mar. 6	July 7	do	0.84	.....
49	Mar. 4	....	do	0.14	Later developed jaundice.
52	Mar. ?	June 18	do	...	Icteric.
SJA	Apr. 17	July 14	July 17	...	do

during the period of jaundice. Vomiting also occurred and varied considerably in severity. Usually vomiting was limited to one or two episodes but in a few instances was persistent, leading to marked dehydration.

There were cases which presented only dark urine, anorexia, vomiting, headache, and pains with no frank jaundice, while other cases remained jaundiced for at least a month. The average individual was jaundiced for about 6 to 10 days. The degree of illness did not appear to be closely associated with the duration of icterus as many who were jaundiced for a considerable period were ambulatory throughout the illness, while some who were jaundiced only a few days were bedridden during the period.

It seems evident from the standpoint of the previous history of immunization, the prolonged incubation period, and clinical symptoms that the disease under observation was identical with that previously described and designated as homologous serum jaundice.

Under the conditions of the outbreak it was deemed wise to limit our collection of possible infectious material to blood or serum which

TABLE IV

SUMMARY OF GROUPS INOCULATED SHOWING INCIDENCE OF JAUNDICE

Group	*No. in Group*	*Inoculum*	*Cases of jaundice*	
			Number	*Per cent*
1	50	Group 1. Lot 331. Yellow fever vaccine in recommended dose. This vaccine contained pooled human serum and is of the same lot which produced jaundice in the United States Army and in the Virgin Islands.	12	24
2	10	Group 2. Pooled serum collected from nine individuals in the Virgin Islands who had received Lot 331 vaccine. Serum was diluted 1:5. Dose 0.5 ml. S.C.* See Table III.	2	20
3	10	Group 3. Lot 367. Dried yellow fever vaccine containing human serum. Heated in 56° C. water bath for 30 minutes before dilution. Given in recommended dose.	2	20
4	10	Group 4. Pooled weekly specimens of serum from a mild case of jaundice in Group 1. Serum dilution 1 : 3. Dose 0.5 ml. S.C.	0	0
5	10	Group 5. Pooled weekly specimens of serum from a mild case of jaundice in Group 2. Serum dilution 1 : 3. Dose 0.5 ml. S.C.	0	0
6	20	Group 6. Pool of same specimens used in Group 2. Serum dilution 1 : 3. Dose 0.5 ml. S.C. See Table III.	6	30
7	20	Group 7. Pooled weekly serum specimens from a severely jaundiced patient in Group 1. Dilution 1 : 3. Dose 0.5 ml. S.C.	3	15
8	20	Group 8. Pooled weekly serum specimens from a moderately jaundiced patient in Group 1. Dilution 1 : 3. Dose 0.5 ml. S.C.	1	5
9	10	Lot 367. Yellow fever vaccine diluted as recommended and heavily irradiated with ultraviolet light, 1 hour at 2650 A. and 1½ hours at 2537 A.	0	0
10	14	Pooled weekly serum specimens taken before appearance of jaundice from patient in Group 1. Serum dilution 1 : 3. Dose 0.5 ml. S.C.	4	28.7
11	15	Group 11. Single serum specimen from same individual contributing to Group 10. Specimen taken about 2½ months after jaundice had subsided. Serum dilution same as in Group 10. Dilution 1 : 3. Dose 0.5 ml. S.C.	0	0
12	10	L. J. Serum. Had contributed serum to pool used in icterogenic vaccine. Had history of jaundice 1 year previously. Dose 0.5 ml. 1 : 4 S.C.	0	0
13	10	M. B. Serum. Description for Group 12. Serum applies here also. Dose 0.5 ml. 1 : 4 S.C.	0	0

(*Continued on p. 437*)

TABLE IV—(*Continued*)

Group	*No. in Group*	*Inoculum*	*Cases of jaundice*	
			Number	*Per cent*
14	11	#38 pooled weekly serum specimens taken during pre-icteric period. Irradiated 45 min., 85% 2537 A. Dose 0.5 ml. 1 : 3 S.C.	0	0
15	13	Same serum used in Group 14, *non* irradiated. Dose 0.5 ml. 1 : 3 S.C.	2	15.4
16	15	Pooled dried plasma. One donor developed jaundice 4 days after bleeding. Dose 1 ml. S.C.	0	0
17	10	Serum from young man during spontaneously occurring jaundice. Dose 1 ml. 1 : 4 S.C.	4	40
18	3	Same serum used in Group 17. Dose 1 .ml. 1 : 4 I.N.†	0	0
19	3	#38 pooled pre-jaundice phase serum. Dose 1 ml. 1 : 4 I.N.	0	0
20	20	#125 pooled pre-jaundice serum irradiated in thin quartz cell 2½ sec. by high energy low pressure water-cooled mercury vapor lamp. Dose 1 ml. 1 : 4 S.C.	0	0
21	9	Same serum as in Group 20 *non* irradiated. Dose 1 ml. 1 : 4 S.C.	1	11
Total	273		37	

* S.C.—Subcutaneously.

† I.N.—Intranasally.

could be shipped under suitable conditions. Many samples were taken and from these nine were selected for experimental use. These are tabulated in Table III.

EXPERIMENTAL STUDY

Volunteers were obtained in an institution with a population of about 1700. During the greater part of the study it was possible to use groups composed equally of both sexes. After 189 persons had been inoculated and 30 had developed jaundice it was found there was no apparent difference in susceptibility of the sexes and thereafter either sex available was used. Individuals with infectious diseases or history of recent antisyphilitic treatment were excluded. The age range was 13-57.

Table IV shows the size of groups used with brief descriptions of materials used for inoculation, and tabulation of cases of jaundice. Groups are arranged in chronological order in the table.

Each serum given was diluted with phosphate-buffered normal saline solution, pH 7.6, Berkefeld-N-filtered and cultured for sterility. Subcutaneous inoculation was always into the arm. Intranasal inoculation was done with the subject lying on the back of a table with the head dependent. Half the inoculum was dropped into each nostril and the subject then sniffed the material well back into the nose. Materials used for inoculation including sera and dried vaccines were stored routinely at 4° C. for variable periods up to a year. The maximum survival time of the icterogenic agent at this temperature was not determined.

All persons inoculated were bled just before inoculation and weekly afterward for 4 to 6 months. Bleeding was done on the same day of the week for each person and at about the same time of day, usually 3-5 hours after breakfast. The serum was separated the same day and a quantitative indirect Van den Bergh estimation was done.[25] Readings were made in a comparator using cobaltous sulfate standards.

Total leukocyte and differential counts were done weekly on the first few groups. No significant variation during the period of jaundice was seen in either count. Schilling counts were then done routinely each week and these failed to show any variation in jaundiced patients.

Moss blood grouping was done for each subject. No correlation between blood group and susceptibility to jaundice was found. Eighty-five sera were tested for Rh factor. Both Rh-positive and Rh-negative individuals were found susceptible to jaundice.

Some groups were skin tested just before inoculation with sera, one of which (Group 17) was icterogenic; 0.1 cc. of serum was given intracutaneously and the test was read 30 minutes later. Some slight reactions with small wheals up to 0.8 cm. in diameter with surrounding zones of erythema were seen. However, there was no correlation between the result of the skin test and susceptibility to jaundice, those subjects developing jaundice being equally divided, two showing small reactions and the other two no reaction. Non-icterogenic sera were also found to give similar slight reactions in some individuals.

The cephalin-cholesterol flocculation test of Hanger[26] was done weekly on each serum specimen, always with fresh serum on the day of bleeding. Invariably the test is strongly positive when clinical jaundice is present. In the subclinical range in our experience, the test may or may not be strongly positive. Many +, ++ and +++ reactions have been seen with sera from individuals who were in normal health so far

TABLE V

SUMMARY OF ALL EXPERIMENTAL GROUPS DEVELOPING JAUNDICE SHOWING INCIDENCE ACCORDING TO AGE GROUP

	Inoculated	*Developed jaundice*	*% developed jaundice*
10 – 14	2	0	0.0
15 – 19	35	3	8.6
20 – 24	45	10	22.2
25 – 29	25	4	16.0
30 – 34	27	8	29.6
35 – 39	17	4	23.5
40 – 44	13	3	23.0
45 – 49	9	3	33.3
50 – 54	2	2	100.0
55 – 59	1	0	0.0
Total.....	176	37	

as known. Difco antigen was used.

Icterogenic serum specimens have been repeatedly examined in the electron microscope by Lt. Don R. Mathieson of the Naval Research Medical Center. No particles of uniform morphology were found.

In Table V are listed all experimental groups in which cases of jaundice developed, according to age groups. The size of the groups is too small to give an accurate idea of the true susceptibility. The survey done in the Virgin Islands is undoubtedly much more reliable in this respect because of the larger number involved.

For statistical purposes jaundice was considered to be present when the serum bilirubin value was 1.0 mg. per cent or higher. Clinical jaundice was usually not seen until this value was 2.0 mg. per cent or more. In Table VI are listed all cases of jaundice occurring in experimental groups with data obtained from weekly blood specimens of each individual. In a few cases subjects were removed from the institution before complete disappearance of jaundice. A short statistical summary of 37 cases of jaundice is given in Table VII. Incubation period is here defined as elapsed time in weeks between inoculation and first appearance of a serum bilirubin of 1.0 mg. per cent or more. Duration of jaundice here

TABLE VI

OBSERVED DATA, CASES OF JAUNDICE ONLY

Group	*No.*	*Age*	*Incubation period, wks.**	*Duration of jaundice, wks.†*	*Time required for serum bilirubin to return to 0.5 mg. %, wks.*	*Highest serum bilirubin observed, mg. %*
1	2	17	14	1	3	5.0
1	7	15	13	2	3	3.0
1	8	24	16	2	3	1.1
1	12	22	14	1	2	1.0
1	13	33	13	3	4	10.0
1	23	46	16	3	3+	8.0
1	30	28	12	3	5	4.5
1	31	21	5	1	2	1.7
1	33	21	12	2	3	4.0
1	38	38	14	6+	6+	20.0
1	44	32	12	1	2	3.0
1	48	47	13	4	5	9.0
2	57	33	15	1	2	1.2
2	59	26	10	2	4	2.2
3	61	42	19	7+	7+	16.0
3	70	37	18	2	3	2.8
6	132	36	12	1	1	2.1
6	136	39	12	1	1	2.0
6	138	22	11	3	3	5.6
6	162	31	12	2	3	1.2
6	163	21	12	3	3+	2.6
6	167	24	10	2	3	4.8
7	150	33	8	2	4	5.6
7	176	21	10	1	2	2.8
7	179	40	10	4	6+	2.6
8	188	32	17	2	3	4.8
10	201	24	13	2	2	2.4
10	208	19	8	1	2	3.2
10	217	26	9	2	4	2.4
10	220	22	10	3	4	8.0
15	112	28	14	3	4	3.3
15	125	43	9	3	4	5.4
17	266	31	20	1	1	2.1
17	271	51	19	3	4	6.2
17	272	46	16	4	5	10.0
17	273	52	20	1	2	2.1
21	306	30	10	2	4	2.4

* Elapsed time until serum bilirubin value of 1.0 mg. % or higher is found.

† Time during which serum bilirubin of 1.0 mg. % or more is found.

TABLE VII

STATISTICAL SUMMARY OF CASES OF JAUNDICE

	Range	*Average*	*Median*	*Mode*
Incubation, period, weeks	5 – 20	12.9	12	12
Serum bilirubin, mg. % (maximum observed)	1.0 – 20.0	4.7	3.0	
Duration of jaundice, weeks*	1 – 7	2.35	2	2
Serum bilirubin elevated, weeks†	1 – 7	3.3	3	3

* Number of weeks during which serum bilirubin value was 1.0 mg. % or higher.
† Number of weeks required for serum bilirubin to return to 0.5 mg. % or less.

means the time in weeks during which the serum bilirubin remained at 1.0 mg. per cent or higher.

In two cases of jaundice a biphasic rise in serum bilirubin was seen. Both cases occurred in group 1. In one case the first rise occurred after 3 weeks and reached 1.1 mg. per cent. In the thirteenth week a second rise to 3.2 mg. occurred with a further rise to 10.0 mg. the following week. In the other case the first rise occurred after 5 weeks reaching 1.8 mg. in the eighth week with a secondary rise to 1.7 mg. in the fourteenth week.

Clinically most cases were quite mild; epigastric discomfort and nausea were commonly present shortly before jaundice appeared. Vomiting occurred in a few cases. Clay-colored stools and dark urine were present during jaundice. Slight fever up to 100°-101° F. was noted in a few cases at about the time of onset. Anorexia was usually present during jaundice. Most jaundiced patients remained ambulatory. Dermatitis and arthritis were not seen.

So far as known no contact cases of jaundice occurred. Only 3 cases have appeared among 1,400 uninoculated individuals not included in this study during the past 18 months in the institution's population of about 1,700. One of these spontaneous cases was found to have carcinoma of the liver. During one period of 4 months 40 uninoculated persons having close contact with the inoculated group in which jaundice was occurring were bled weekly and no evidence of jaundice was found among them.

Colonel Stanhope Bayne-Jones supplied sera from three persons who

had acted as donors to pools of serum used in preparation of batches of yellow fever vaccine which had produced jaundice in the United States Army during 1942. It was subsequently found that all three of these persons had histories of jaundice occurring several months before they were bled for the vaccine. Two of these sera were used for inoculation in groups 12 and 13. No cases of jaundice appeared in these groups.

The plasma used in group 16 was a part of one lot prepared commercially. Before the lot was released it was learned that one of the donors to the pool had developed jaundice a few days after being bled. The whole lot was immediately confiscated. On July 22, 1943 a group of 15 were inoculated with 1 cc. each of this material subcutaneously. No cases of jaundice developed. On December 10, 1943, another group of 10 were inoculated with the same material in a dose of 10 cc. intravenously. To date no jaundice has appeared in the second group.

The serum used for inoculation in group 17 was obtained from a boy 18 years old, an inmate of the institution, who developed jaundice spontaneously. He had had no known contact with members of the experimentally inoculated groups. Serum was obtained from him five days after jaundice had appeared, at which time he complained only of poor appetite; there was no fever, the serum bilirubin value was 12 mg. per cent; the Hanger reaction was strongly positive. This case and the 4 cases which subsequently developed in those inoculated from it were indistinguishable in appearance from the other cases seen. The incubation periods ranged from 16 to 20 weeks.

Reports of two cases of jaundice among the personnel of one Coast Guard vessel were received in 1943. Both men were vaccinated the same day with one lot of "aqueous base" yellow fever vaccine containing no serum. One developed jaundice in forty-nine days, the other in 129 days. The findings in both cases, as reported, were consistent with the finding in cases of our series. On November 11, 1943, thirteen subjects were inoculated with twice the recommended dose of the same lot of vaccine mentioned above as used on the Coast Guard vessel. To date no cases of jaundice have developed in this group.

Attempts were made to transmit jaundice to animals, using materials which had produced the disease in humans. These included yellow fever vaccine as well as sera derived from patients in the Virgin Islands and from persons with experimentally produced jaundice. Monkeys, pigs,

rabbits, guinea pigs, white rats, Swiss mice, cotton rats and Syrian hamsters were employed. In no instance were we able to produce any illness in experimental animals which could not be accounted for by other agents and no animals became jaundiced as determined by physical or chemical examinations.

E. W. Goodpasture supplied specimens of liver from fatal cases of jaundice which occurred following the use of yellow fever vaccines. Extracts of these specimens were made both with 95 per cent alcohol and normal saline solution. The extracts were used as antigens in complement fixation tests designed to determine whether or not serum antibodies were present following this type of hepatitis. In a limited series of tests negative results were obtained. Attempts were also made to produce a complement-fixing antigen in the developing chick embryo; these were inoculated both in the allantoic sac and the yolk sac. Embryos 9-10 days old were inoculated with icterogenic yellow fever vaccine. Serial transfers were made in both series at intervals of 3-4 days and at 1 week. No fixation resulted with allantoic fluid or yolk sac emulsions tested against human sera obtained after recovery from jaundice.

Comment

It was recognized in 1942 during an epidemic of jaundice in the United States Army that some agent in human serum employed as a diluent in yellow fever vaccine was probably responsible. The yellow fever vaccine now in use does not contain serum. The two cases described above of jaundice in personnel of the Coast Guard following administration of serum-free vaccine may well have been due to other causes than the vaccine and probably should be so regarded unless evidence to the contrary is obtained.

It is difficult to evaluate the claim of Findlay and Martin[16] to have produced jaundice in 3 of 4 subjects inoculated with nasal washings of patients in the early stages of post-yellow fever vaccine jaundice. Two of these cases are claimed to have been subclinical jaundice. In one, T.P., the icteric index did not exceed 8 units. In the other, M.M., the only direct evidence of jaundice was a weakly positive direct reaction (serum Van den Bergh). So far as known no cases occurred among familial contacts of thousands of cases of jaundice occurring in the U. S. Army and it is felt that additional evidence should be obtained before concluding that the condition may be transmitted by the respiratory tract.

Since jaundice has repeatedly followed the administration of whole blood or blood products there is an urgent need for some means of 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.

Summary

Results of a sample survey of an epidemic of jaundice occurring subsequent to vaccination against yellow fever in the Virgin Islands in 1942 are given.

Jaundice was produced experimentally: 1. By the inoculation of two lots of yellow fever vaccine containing human serum. 2. By the inoculation of small amounts of filtered serum from each of three individuals and of a serum pool from nine individuals all of whom had previously received yellow fever vaccine containing human serum. 3. By inoculation of serum from one individual who had early spontaneously occurring jaundice.

Two sera which were icterogenic when inoculated subcutaneously failed to produce jaundice by the intranasal route.

Both sexes are apparently equally susceptible to this type of jaundice. Those of all four Moss blood groups and both Rh-positive and Rh-negative persons were found to be susceptible.

Susceptible persons did not give uniform local skin reactions to icterogenic sera.

The jaundice-producing agent is filterable, survives drying in vacuum, storage for long periods in serum at 4° C. and heating to 56° C. for one-half hour in the dried state. The agent was found to be present in the blood during the pre-jaundice period but not 2½ months after the disappearance of jaundice.

The icterogenic agent is apparently inactivated by short exposure to ultraviolet irradiation.

Transmission of jaundice by ordinary contact apparently did not occur during this experiment.

Attempts to produce jaundice in experimental animals were unsuccessful.

Antigens prepared from human livers and from chick embryos failed to fix complement in the presence of sera obtained after recovery from jaundice.

Attempts to visualize virus particles in icterogenic sera by electron microscopy were unsuccessful.

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