S. ARMY EPIDEMIOLOGICAL BOARD

REPORT OF MEETING OF THE COMMISSION
ON NEUROTROPIC VIRUS DISEASES

Report of Meeting of

THE COMMISSION ON NEUROTROPIC VIRUS DISEASES

of the

U.S. Army Epidemiological Board

held at

The Rockefeller Institute for Medical Research

New York City

18 October 1945

Owing to the limited time available at the meeting some of the reports listed in these minutes were "read by title". They are marked with an asterisk.

The meeting was called to order at 10:00 AM at the Rockefeller Institute. The following were present:

Members of the Commission.- Drs. C. D. Aring, G. D. Gammon, W. McD. Hammon, Maj. W. P. Havens, Jr., Dr. C. A. Janeway, Lt. Col. A. B. Sabin, Capt. R. W. Schlesinger, Dr. R. Ward, and Dr. J. R. Paul, Director.

"Associate Members".- Drs. P. K. Olitsky and J. Casals.

Representing the Surgeon General's Office.- Lt. Col. Aims C. McGuinness.

Representing the Army Medical School.- Col. H. Plotz, Lt. Col. J. E. Smadel, Lt. J. Warren, Maj. L. Whitman.

Guests.- Drs. D. M. Horstmann, H. A. Howe, K. F. Maxcy, J. L. Melnick, Mr. J. T. Riordan, Maj. E. B. Schoenbach, Dr. M. Theiler.

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INTRODUCTION, by Dr. J. R. Paul.

In thanking members and guests for coming, Doctor Paul stated that this might be the last formal meeting of the Commission. A brief account of some of the accomplishments of the group during the past three years was reviewed, with an expression of appreciation and pride in the Commission's work during the war years.

FUTURE PROGRAM, by Lt. Col. A. C. McGuinness, M.C.

Colonel McGuinness spoke briefly on postwar plans, stating that the Army Epidemiological Board would eventually become a part of the recently organized Army Medical Department Research and Development Board. Present plans for the postwar Army Epidemiological Board call for a Central Board composed of five members, and in addition about twenty Consultants. There will be no permanently organized Commissions as at present. Commissions will be constituted from time to time for investigation of special diseases and epidemiological problems, and membership on Commissions will change from time to time as need may indicate. While a budget for contracts with universities is contemplated, nothing definite has been done about this as yet, and he advised that we should not count on having Army funds after 1 July 1946.

1. ENCEPHALITIS OUTBREAK ON OKINAWA by Lt. Col. A. B. Sabin, M.C.

History of Outbreak.- The first known civilian cases of encephalitis had their onset about 1 July 1945, and were detected between 7 July and 9 July, both on the mainland of Okinawa and on the adjacent island of Heanza. By the end of July at least 80 cases had been found among civilians of whom 22 died, but only 3 suspected and 3 probable cases (only one of which has been confirmed by serological tests subsequently) among American military personnel. On the isolated islands of Heanza and Hamahika the epidemic apparently terminated by the end of July with an incidence of 4.7 per 1,000 (31 cases among a total

population of 6,547) and 3.3 per 1,000 (5 cases among 1500) respectively. The actual incidence of cases on the mainland of Okinawa has remained difficult to determine since it has been estimated that only 1/3 to 1/6 of the total number of cases was detected. During the month of August an additional 35 to 40 civilian cases were found on Okinawa, and 18 to 20 probable cases with 2 deaths occurred among American military personnel. A small number of additional civilian and military cases occurred during September. The death rate among civilians was about 25 per cent despite the fact that only the moderately severe and severe cases were detected.

Approximately 80 per cent of the civilian cases (94 of 118) occurred in infants and children 15 years of age or younger. Native physicians indicated that "summer encephalitis" predominantly among the younger age groups is an annual occurrence on Okinawa usually with an incidence of 5 per 10,000, although one year was recalled in which the incidence was 17 per 10,000.

Identification of Etiological Agent.- The etiological agent of the outbreak was identified as the Japanese B encephalitis virus by complement fixation and neutralization tests on convalescent sera and by isolation of a single strain of the virus from the brain of a fatal civilian case. This work was done by members of the Naval Medical Research Unit No. 2 (NAMRU No. 2) and myself. Many attempts were made to isolate virus from the blood and cerebrospinal fluid of civilian and military cases but with consistently negative results. Isolation of the virus from human brain tissue was also difficult since no virus could be isolated from certain cases which exhibited typical lesions.

Clinical Manifestations.- The clinical manifestations corresponded in general to previous descriptions of Japanese B encephalitis and were not unlike those seen in the summer encephalitides in the U.S.A. "Eye signs" (dissociated eye movements, strabismus, diplopia, nystagmus, pupillary changes) were not at all infrequent, however, in this outbreak.

Pathology.- Studies were made of the brain and cord of the fatal Army case and Lt. Comdr. Zimmerman of NAMRU No. 2 examined tissue from a number of civilian cases and from one marine. The extensive involvement of the Purkinje cells in the cerebellum was perhaps the least expected finding. Otherwise the nature and distribution of the lesions were compatible with the diagnosis of encephalitis. No such Purkinje cell lesions were found in the brains of two mice inoculated intracerebrally with the Okinawa strain of virus.

Entomological Aspects.- The most prevalent mosquito in the encephalitis foci was Culex quinquefasciatus. Rare specimens of Armigeres subalbatus were taken and a few larvae, but no adults, of Aedes togoi were also found. The NAMRU No. 2 entomologists also collected some Culex bitaeniorhynchus and Aedes albopictus on Heanza, and Culex tritaeniorhynchus on the northern end of Okinawa. Several attempts to isolate virus from pools of Culex quinquefasciatus yielded negative results; the more extensive tests carried out with this and other species of mosquitoes by the NAMRU No. 2 team have also yielded negative results.

Search for Ectoparasites and Extrahuman Hosts.- No mites were found on the few chickens which remained on the island. Two pools of Trombidiid mites were tested by mouse inoculation--one collected for me by Capt. Parsons, Sn.C., from a Heanza dove, and another derived from doves and other birds on Heanza collected by the NAMRU No. 2 group--with negative results. No mites were found on the horses, goats, or pigs which were examined. A few parasitid mites (not yet identified further), not enough for a test, were found on a few rats by Capt. Parsons, as well as the NAMRU No. 2 entomologists. A number of neutralization tests have already been completed on the sera obtained from chickens, horses, and goats, and others are in progress. Neutralizing antibodies for the Okinawa strain of Jap. B encephalitis virus have already been found in the horse and goat sera but not in the chicken sera. Lt. Horace Hodes, USNR, of NAMRU No. 2 has informed me that he has obtained positive

complement fixation tests with each of 5 horse sera from Okinawa but not with the serum of a horse from Guam, used for control.

Some Epidemiologic Features.- It is perhaps noteworthy that the cases of encephalitis were limited to the northern portion of Okinawa to which almost the entire civilian population had been moved. Although less than a third of all the military personnel were in this northern area, all the encephalitis cases occurred among them, while not a single definite case was reported among the remaining 2/3 or more of the forces occupying southern Okinawa, which was not vaccinated and which was no more free of the prevalent mosquitoes. It almost seemed as if the reservoir of the virus had been moved with the native population or the domestic animals which accompanied it. The outbreak followed the usual climatic aspects, i.e., there were heavy rains in April and May followed by at least 4 to 5 weeks of a very hot, dry spell before the first cases appeared early in July. Most of July continued hot and dry but ended with heavy rains, while August was stated to have had more rain than usual. What we looked for, and did not find, was direct evidence for the working hypothesis that a mite-like insect, constituting the real reservoir of the virus, gives rise to viremia in some of the domestic animals, with subsequent dissemination to human beings and other hosts by the mosquitoes which breed in large numbers during the hot spell following the rainy season. This may still be the true epidemiologic cycle despite the fact that neither the "mite" nor the mosquito which might have carried this virus have as yet been found on Okinawa. Considering the unusually crowded conditions under which the natives lived, there was little to favor any concept of direct human to human spread of the encephalitis.

Neutralizing Antibodies in Native Okinawans of Different Ages.- 62 sera from individuals of 6 different age groups ranging from 1 to 61 years were collected for tests as a check on the hypothesis that Okinawa is an endemic area and that most of the adult population had already had the disease. The neutralization tests on these sera using the Okinawa strain of virus are

now in progress, and one test which has gone far enough shows the absence of antibody in at least 9 of 10 sera from the 1 to 5 age group as contrasted with the probable presence of antibody in 8 of 9 sera from the 31 to 51 age group.

Neutralizing and Complement Fixing Antibodies in Sera of Military Personnel with a Clinical Diagnosis of Encephalitis.- These tests are still in progress but there is already an indication that no neutralizing antibodies are demonstrable in the convalescent sera of some of the severe as well as mild cases, clinically diagnosed as encephalitis, although they have been demonstrated in others.

Vaccination Program.- Although 800 to 1,000 of the most exposed military government personnel were vaccinated between 15 and 20 July, the decision to vaccinate the military personnel stationed near civilian foci of infection was reached on 1 August at a time when the future course of the outbreak could not be predicted. It was not deemed advisable to gamble on the chance that very few cases and deaths would occur among the military personnel, or to rely entirely on mosquito control measures. Enough vaccine had been distributed to inoculate over 82,000 individuals. Because of the great mobility of units on the island and the multiple services and commands involved, the exact data, which are being collected by Major W. G. Downs, are not yet available, but it is known that at least 50,000 and probably 65,000 of the approximately 75,000 military personnel occupying the portion of Okinawa, north of Ishikawa, were vaccinated during the month of August. No serious reactions have been reported from the vaccine. There were a small number of transitory allergic manifestations characterized by asthmatic-like reactions, urticaria, or angioneurotic oedema. The reports are not all in yet but the incidence of these "allergic" manifestations may be judged by the occurrence of only one such reaction (asthmatic and urticarial) among a group of approximately 5,000 marines. There were also a certain number of local inflammatory and short febrile reactions. No case suggestive of a postvaccinal demyelirating

encephalopathy has been reported to date. Two very mild cases of encephalitis had their onset 2 and 6 days respectively after the first dose of vaccine. After 1 September there were 4 mild cases, whose chief neurological manifestations were nuchal rigidity, varying degree of pleocytosis, and transitory reflex changes, in individuals who had their first dose of vaccine 15 to 18 days before onset. If the complement fixation tests on these patients become positive in the convalescent sera it will be possible definitely to class them as mild cases of encephalitis. Otherwise the etiological diagnosis will remain equivocal. I do not think that it will be possible to arrive at any conclusion regarding the role the vaccine might have played in keeping down the number of cases of encephalitis among the military personnel, despite the fact that not a single severe or moderately severe case of encephalitis occurred in any vaccinated person.

2. OKINAWA ENCEPHALITIS INVESTIGATIONS by Dr. W. McD. Hammon.

A review of Dr. Hammon's Okinawa survey trip with Dr. Reeves can be given briefly.

Arrival in Okinawa, 26 August. Headquarters at Kana at the Military Government Encephalitis Research Hospital for about one week during which time native cases which were hospitalized there were seen. Epidemiological data were reviewed and preliminary observations made on mosquito population. Mosquitoes had been practically eliminated on that section of the coast so another area was investigated at Nago with the 87th Field Hospital. These headquarters were kept until the time of leaving Okinawa on 20 September.

The report of the studies undertaken which included collection of sera from military and native cases, from local animals and birds, collection of mosquitoes and human autopsy material has been filed with the records of the Neurotropic Virus Disease Commission as of the date of 9 October 1945. Subsequent and new data ~~is~~ are as follows.

Mosquitoes.- Half of the Culex quinquefasciatus collected have been tested for virus. No virus has been detected. This was not wholly unexpected as the mosquitoes were collected after the subsidence of the epidemic. It has been the experience in the Yakima Valley with Western equine encephalomyelitis and St. Louis encephalitis viruses that infected mosquitoes are found early in the summer before the outbreak in human beings and not later on.

Sera of Military Cases.- A number of neutralization tests against the Jap B virus have been performed, using the least amount of virus which will kill all mice with fair regularity. Very few convalescent military cases gave a slight degree of protection, of no significance in most instances. However, when a few of these together with other Okinawa sera were tested with our chick embryo antigen for complement fixation (C.F.), it was observed that those without any demonstrable neutralizing antibody were negative and most of those with very slight protection fixed complement, 2 of them in titers of 1:32 and 1:64. Sera from 7 convalescent native cases were also tested by both methods. The undiluted serum of 5 (#74,7,65,86 and 90) showed at least some slight protection, and had C. F. titers of 1:4 to 1:32, while the other two were entirely negative by both tests. As a result of the complement fixation tests it is felt that the very low neutralization test titers may possibly be significant. Even with this generous interpretation only 7 of the 22 Military "cases" from which sera were obtained gave serological evidence of infection with Jap B virus. Two of these had been vaccinated and showed no increase in neutralizing titer between 2 specimens collected, one (Liacey), showed C.F. of questionable significance in both specimens, the other (Brogan) gave a titer of 1:16 in the 8 day serum but the later serum gave unsatisfactory results with a control antigen.

Sera of Vaccinated Military Personnel.- Of 15 vaccinated individuals tested for neutralizing antibodies, only 1 was positive. The latter had been stationed in an area containing many native cases, and had developed also a C.F. titre of 1 to 4. These data suggest that vaccination as performed on

Okinawa did not give rise to neutralizing antibodies in a high proportion of individuals.

Native Cases.- Of 15 convalescent sera from native cases tested for neutralizing antibody, 7 had neutralizing antibody to a significant degree. As mentioned above, 7 that were checked by C.F. correlated perfectly with no protection or with minimum detectable protection. No serial sera on any native cases were tested to rule out the presence of Jap B antibody in the acute phase sera.

Normal Native Sera.- Some of the normal native sera of the age groups up to 20 have been tested for neutralizing antibodies. The results are as follows:

AGES	No. LD_{50} doses neutralized			
	10		50	
	Pos.	Neg.	Pos.	Neg.
0 - 4	0	7	0	7
5 - 9	1	7	0	8
10 - 14	6	1	4	3
15 - 19	5	0	2	3

It may be tentatively concluded from these small numbers that few natives under 10 years of age have been previously infected and that in the age group from 10 to 20 infection has occurred in a considerable proportion.

Commenting on Col. *Sabin's* statement that he (Sabin) had been unable to detect virus in blood or spinal fluid of patients, Dr. *Hammon* suggested that many of the samples of blood and spinal fluid may have been taken from patients not having Jap B encephalitis.

Col. *Sabin* answered that 6 of 7 cases proved serologically as Jap B encephalitis had failed to yield virus in the blood or spinal fluid.

Col. *Smadel* asked why complement fixing antibody titres were so high when neutralizing antibody titres were relatively low. He raised the question of whether the subjects had also received typhus vaccine because the sera of 20% of troops receiving "booster" doses of typhus vaccine developed complement fixation with normal chick tissue. Dr. *Hammon* replied that no

fixation was obtained with normal chick embryo used as control antigen.

Col. Sabin observed that none of Hammon's group of patients included early July cases; nevertheless even certain of the latter considered as good examples of Jap B encephalitis failed to show neutralizing antibody.

3. PREPARATION OF JAPANESE B ENCEPHALITIS VACCINES FROM THE INFECTED CHICK EMBRYO by Lt. J. Warren, Sn.C.

In this the second report to be presented before the Commission on this subject, progress is described with the method of preparation of a chick embryo vaccine against Japanese B encephalitis. Preliminary tests of this vaccine in the Virus Laboratory at the Army Medical School and at the National Institute of Health, indicate its potency to be comparable to the present commercial mouse brain vaccine.

Preparation of Vaccine.- The seed virus used in the inoculation of eggs was harvested and stored as a 10% suspension of infected embryo in whole filtered chicken serum. Such seed suspensions withstood storage at -70°C in sealed ampules for more than one month with no appreciable loss of titer.

Both the seed virus and the material for vaccine was obtained from embryonated eggs which were 7 or 8 days old when infected via the chorio-allantoic sac. These eggs were subsequently incubated at 35°C for three days at which time the embryos were harvested.

The whole embryo was removed from groups of 20 to 200 infected eggs. The lens and retinal cup were dissected out and discarded. The remaining embryo tissue was then homogenized in a Waring blendor for 5 minutes with sufficient physiological saline solution, buffered at pH 7.4 to make a 10 or 20 per cent suspension by weight. The resulting suspension was strained through gauze. After samples were removed for sterility tests and for titration of infectivity, sufficient formaldehyde U.S.P. was added to give a final concentration of 0.2%. The material was then placed in the refrigerator

for two weeks, following which the vaccine was assayed for potency.

All of the chick vaccines have been assayed in the same manner as that used for commercial mouse brain vaccine, namely the mouse protection test devised by Sabin and Duffy.

Results.- In order for a vaccine prepared from the embryonated egg to merit consideration for human use it must satisfy the standard of potency accepted for a mouse tissue vaccine. This requirement states that a vaccine may be regarded as of acceptable potency if the minimal immunogenic dose protecting 50% of the mice (M.I.D. 50%) is 0.01 cc. or less.

The lowest M.I.D. 50% which we could obtain with a 10% embryo vaccine was 0.03 cc. This vaccine had an initial infective titer of $10^{-7.5}$. By centrifugation at high speed in the Sharples or air-driven angle ultracentrifuge it was possible to achieve a 2 to 3 fold increase in potency. However this concentration of antigen was obviously still insufficient. It was then found that by increasing the concentration of infected embryo to 20%, vaccines could be prepared which would meet the accepted requirements. Thus, 8 of 11 twenty per cent vaccines assayed between 0.002 cc. and 0.01 cc. There is some correlation between the titer of virus in the vaccine before inactivation and its potency. In general, infective titers of $10^{-7.5}$ or greater are required for a successful vaccine.

Attempts to concentrate the chick embryo vaccine on calcium phosphate or potassium alum met with failure. On the other hand the potency of these preparations could be increased two or three-fold by the use of an adjuvant consisting of equal parts "Falba" and mineral oil. In these experiments a 2% suspension of adjuvant in physiological saline was used as the diluent in making the vaccine assay. Because "Falba" cannot be used in the vaccination of man we are at present testing related but less harmful compounds, for any similar adjuvant activity.

No tests of these embryo vaccines have as yet been performed in man.

Discussion.- Col. Sabin mentioned 2 factors to be considered in deciding what type of vaccine should be prepared for the summer of 1946: 1.) Commercial houses are already making mouse brain vaccines. 2.) How desirable is it to give another chick embryo vaccine to individuals already vaccinated with chick material?

Col. Smadel observed that the response to chick embryo Jap B vaccine in human beings is unknown.

Col. Plotz stated that the amount of egg white in the chick embryo was much less than that in the yolk sac. He reported that there had been 14 serious reactions to chick embryo vaccines and some deaths.

4. DIPHTHERITIC POLYNEURITIS by Dr. G.D. Gammon and Maj. E.B. Schoenbach, M.C.

A group of 70 cases of multiple peripheral neuritis among German Prisoners of War was studied. The chief problem was to determine what proportion were post-diphtheritic. A careful history was taken including the German laboratory data and treatment, and a comprehensive neurological examination made. From this it was found that the cases fell into several groups which had characteristic clinical syndromes; a few did not fit into these definite categories. The division was as follows: 1) Diphtheritic neuritis, 42; 2) sulfonamide neuritis, 13; 3) post-infectious neuritis from wound infection with severe general sepsis, 6; and 4) unclassified cases, 9.

All of these, and a group of control cases, were cultured on two successive days for C. diphtheriae from nose and throat, and serum antitoxin levels were determined by the method of Fraser. The controls consisted of 112 individuals without diphtheria and 17 cases of recent diphtheria. The total group comprised 199 individuals.

The outstanding finding was that 17% of the normal controls carried virulent C. diphtheriae. This exceeded, though not significantly, the positives in the diphtheritic neuritic group. Between the various neuritides there was no significant difference in the percentage of positives. This

bacteriologic study, therefore, did not differentiate diphtheritic neuritis from other types.

Antitoxin levels in serum were lower in the cases of recovering diphtheria and in the diphtheritic neuritis cases than in the control group. But between the different neuritides there was no significant difference. This test, therefore, also failed to distinguish diphtheritic neuritis from the other types.

The failure of the culture method to differentiate is probably due to two causes: 1) the very high carrier rate and, 2) the fact that the neuritis cases studied late at a time when organisms were disappearing from the nose and throat and the group was approaching the positivity of the surrounding community. In both tests the small size of the non-diphtheritic groups militated against differentiation.

Despite these negative results, on clinical grounds, the various types were considered distinctive. Thus, post-diphtheritic neuritis was characterized by 1) slow development of the syndrome occurring 4 to 6 weeks after onset of diphtheria, and 2) paralysis of palate and pupil accommodation. Sulfonamide (methyl sulfathiazole or "Vleron") neuritis was characterized by sudden onset of paralysis in the legs a few hours after taking the drug, no sensory involvement, by paralysis and wasting of the opponens pollucis and interossei, and no recovery after 6 to 8 months.

CLINICAL CLASSIFICATION OF POLYNEURITIS				
Total Polyneuritis				70
1. Diphtheritic neuritis			42	
Certified dip., typical neuritis		21		
Probable dip., typical neuritis		14		
Skin dip., typical neuritis	5			
Probable dip., incomplete neuritis		7		
2. Sulfonamide neuritis			13	
3. Post-infectious neuritis (Wound sepsis)			6	
4. Unclassified neuritis			9	
No sore throat, atypical neuritis		5		
Sore throat, incomplete neuritis		4		
CONTROLS				112
Recovering diphtheria				17
More than 4 weeks after serum	8			
Less than 4 weeks after serum	9			
TOTAL				199

October 15, 1945

Clinical Classification and Results of Cultural and Serologic Studies of Polyneuritis Cases at Merano, Italy, July 1945

Clinical classification by Dr. Gammon	No. studied	No. cultured	Culture + virulent C diphtheria		Antitoxin content of serum				Cult. neg. for vir. C diphtheria. Antitoxin content of serum			
					<.01 unit		>.01 unit		<.01 unit		>.01 unit	
			No.	%	No.	%	No.	%	No.	%	No.	%
Diph. Polyneuritis	42	42	5	11.9	23	54.6	19	45.4	22	59.6	15	40.4
Sulfonamide Polyneuritis	13	13	0	0.0	5	38.4	8	61.6	5	38.4	8	61.6
Post-infectious Polyneuritis	6	6	2		2		4		2		2	
Unclassified Polyneuritis	9	9	3		3		6		3		3	
Total Polyneuritis	70	70	10	14.2	33	48.6	37	51.4	32	53.4	28	46.6
Control Group No Diphtheria No Polyneuritis	112	107	18	16.9	25	22.4	87	77.6	20	22.4	69	77.6
Convalescent Diphtheria	17	17	9	52.6	5		12		1		7	
a) More than 4 weeks after therapy	8	8	4	50.0	4		4		1		3	

5. VACCINATION OF HUMAN BEINGS WITH DENGUE VIRUS MODIFIED BY PASSAGE IN MICE

by Lt. Col. A. B. Sabin, M.C. and Capt. R. W. Schlesinger, M.C.

It was reported at the last meeting of the Commission that the results of tests on 9 human volunteers inoculated with the 7th to the 10th mouse passage virus indicated that the virus had undergone sufficient attenuation to permit its consideration for use as a vaccine against dengue. During the past 6 months two vaccines, prepared respectively from 15th and 19th mouse passage virus, were tested both in ASTP medical student volunteers and schizophrenic patients. The 15th passage vaccine, consisting of a centrifuged 1:10 mouse brain extract in 10 per cent human serum-saline solution, was tested in 10 students and 6 schizophrenic patients in doses of 0.5 cc. of the 1:100, 1:1,000, and 1:10,000 dilutions. All of these developed a maculo-papular eruption of varying extent, while systemic symptoms were either absent, negligible or very mild; all were found to be immune upon exposure to Aedes aegypti mosquitoes of proved infectivity 21 to 38 days after vaccination. It was thus found that the extract from a single mouse brain and cord preserved in the frozen state had enough antigen in it to immunize at least 10,000 people. When some of the same 15th passage vaccine was lyophilized together with yellow fever vaccine and tested in 10 students, it failed to produce immunity in at least half of those who were exposed to the infected mosquitoes. An extensive test was carried out to determine the infectivity of Aedes aegypti mosquitoes feeding on people from one to 14 days after vaccination with the modified dengue virus. The engorged mosquitoes were allowed an extrinsic incubation period of 29 to 33 days before different pools representing different days after vaccination were tested on 14 students. With 22 to 42 mosquitoes engorging in the tests on each volunteer, there was no evidence of dengue infection in any of the 14 men.

Since it was desirable to get away from human serum as a constituent of the vaccine, crystalline bovine albumin was selected as the protein to be used for both extraction and lyophilization in the tests with the 19th mouse passage

vaccine. A relatively large amount of vaccine was prepared both as a model for production on a still larger scale and for the purpose of having enough available for a field trial if the preliminary human tests were satisfactory. The vaccine was lyophilized in two forms--as 10 per cent and as 1 per cent extract of mouse brain and cord in 10 per cent crystalline bovine albumin. Simultaneous titrations in mice of the frozen and lyophilized 10 per cent vaccines revealed that the lyophilized material had only 1/10 as much virus as the frozen preparation. Human tests on 15 subjects (8 students and 7 schizophrenic patients) revealed that the 10 per cent lyophilized vaccine produced the same type of reactions as the 15th passage frozen vaccine except that in the 1:10,000 dilution, the lyophilized preparation produced rash in only 1 of 3 subjects and immunity to a large dose of unmodified dengue virus (perhaps as much as 1,000,000 human M.I.D.) in 2 of the 3 subjects. The 1 per cent lyophilized vaccine was apparently ineffective since neither rash nor immunity resulted from the 1:1,000 or 1:10,000 doses.

It is believed that this 19th mouse passage lyophilized dengue virus is suitable for a trial of its protective capacity during an epidemic of the disease. Enough of this vaccine is now on hand to permit such a trial on a large scale.

6. <u>DENGUE VIRUS IN CHICK EMBRYOS</u> by Capt. R. W. Schlesinger, M.C. and Lt. Col. A. B. Sabin, M.C.

We have previously reported that various attempts to propagate unmodified dengue virus in embryonated eggs or tissue culture media yielded negative results. More recent tests in which the mouse-adapted Hawaiian strain was used as starting inoculum can be summarized as follows:

1. Attempts to propagate the mouse-adapted virus, using passages IV, V and XIII, in fluid or plasma clot cultures containing mouse embryo brain tissue were unsuccessful in that no virus pathogenic for mice was demonstrable after one, two or three passages.

2. When the 13th passage mouse-adapted virus was used for inoculation of 6- or 10-day old chick embryos which were subsequently incubated for 8 or 4 days respectively at 37°C, no mouse-pathogenic virus was demonstrable after one to four serial passages. The results were the same when the inocula were introduced into the yolk sac or embryo, allantoic or amniotic sacs.

3. When the 13th passage mouse-adapted virus was inoculated directly into the brain of 10-day old embryos which were subsequently incubated for 7 days at 37°C, mouse-pathogenic virus was demonstrable in the chick embryo brain tissue in the first passage, but not in the second. Repetition of this procedure with 16th passage mouse-adapted virus yielded negative results even in the first passage.

4. When the 16th or 18th passage mouse-adapted virus was used for inoculation (toward the embryo) of 5-day old chick embryos which were subsequently incubated for 8 days at 37° C, mouse-pathogenic virus was demonstrable in whole embryo extract of the first passage in 3 separate series. In only one of these series, however, was virus demonstrable in the second passage, but even in this series it was no longer present in the 3rd passage when the incubation was at 37°C. It was found, however, that incubation at 35°C was more suitable for the serial propagation of the virus, so that it has been possible to demonstrate appreciable amounts of virus in the 3rd passage of at least 2 series. There is some indication that 4 or 5 days of incubation is not as good as 8 days, and that while the virus is present in the amniotic membrane as well as in the whole embryo, little or no virus has been found in the amniotic fluid.

5. The pathogenicity of this chick-embryo passaged virus for human beings is now under investigation.

6. The results of the tests on human beings which became available several days after the meeting, indicated that the chick embryo material which was pathogenic for mice, produced the same clinical manifestations as the mouse passaged dengue virus.

7. DENGUE NEUTRALIZATION TESTS IN MICE. by Capt. R. W. Schlesinger, M.C. and Lt. Col. A. B. Sabin, M.C.

The presence of type-specific neutralizing antibodies in dengue convalescent sera demonstrable by tests on human beings was previously reported to the Commission. When the mouse-adapted virus had attained sufficient virulence to produce clinically apparent infection in almost all mice inoculated with a 10 per cent mouse brain suspension, investigations were undertaken to determine whether or not neutralization tests could be carried out in mice. It has been found thus far, that when five to ten 50 per cent infective doses (ID_{50}) are used, the neutralizing effect is demonstrable by absence of clinical signs and survival of the mice, whereas with larger amounts of virus the only effect may be a prolongation in the incubation period. The marked dependence of the test on the quantity of virus used has caused considerable difficulty especially since the virus has been increasing in titre recently. The results obtained thus far indicate that, as in the human tests, the neutralizing effect of dengue convalescent serum in mice is type-specific. In its present stage of development the mouse test seems, however, to be much less sensitive than the human test. Further work is necessary to determine the best way to carry out the test, especially with more virulent mouse virus, before any decision can be reached regarding its possible use in diagnosis or epidemiological investigations.

8. REPORT OF COMMITTEE ON NEUTRALIZATION TESTS by Dr. P. K. Olitsky.

Dr. Olitsky stated that his Committee had considered the inadequacy of the directions issued by the previous committee on neutralization tests and had discussed at some length methods of improving the situation. He believed that owing to the complexity of the problem and the fact that the tests vary greatly with different viruses it now seemed unwise for the Commission to champion any special technique unless there was a demand on the part of certain Army laboratories for such directions.

Col. Smadel inquired as to whether the Commission might not advise their laboratory on certain features which have been found to influence the tests, even though no techniques were recommended specifically.

It was decided to inquire from the Surgeon General's Office for advice as to whether a demand for directions in the performance of the neutralization test now exists.

*9. THE CURRENT STATE OF PRODUCTION OF EQUINE ENCEPHALOMYELITIS VIRUS VACCINES

by Dr. P.K. Olitsky.

The Lederle Laboratories are now processing 30 liters of Western and 30 liters of Eastern virus vaccines for human use. However, more than 100 liters of veterinary vaccines are available. It is the opinion of Dr. Cox of the Lederle Laboratories that, in the face of sudden need for mass immunization, this vaccine can be diluted 1:1 and used for man. The veterinary vaccine differs from the human in that it contains 40 per cent tissue instead of 20 per cent; has 0.4 per cent formalin instead of 0.2 per cent; is not centrifuged, and has not the phenyl mercuric nitrate (1:12,500) used in the human product. But the same "identity", potency and sterility tests are applied to both types.

A current project is the determination of a means for increasing the stability of the vaccine since its immunogenic potency begins to fall after 8 to 10 months' storage.

*10. RUSSIAN SPRING-SUMMER ENCEPHALITIS. FINAL REPORT ON VACCINATION STUDIES

by Drs. J. Casals and P.K. Olitsky.

Continuing studies on resistance to infection and antibody response following vaccination with Russian Spring-Summer (Tick-Borne) encephalitis virus [see report of Commission Meeting April 3, 1945] show 1) mice about 1 1/2 years old exhibited staunch immunity to peripheral (but not to intracerebral) inoculation of virus (to >8 million lethal doses) about 15 months

after vaccination; 2) neutralizing antibody was then still high (serum neutralized more than 30,000 m.l.d.), as determined by intraperitoneal test but practically negative by the intracerebral route (neutralization of 8 m.l.d.); 3) complement-fixing antibody was not detectable.

A correlation therefore exists in mice between neutralizing antibody as determined by the intraperitoneal test and immunity to peripheral inoculation of the virus.

*11. EFFECT OF NORMAL SERUM ON TITER OF VIRUS by Drs. J. Casals and P.K. Olitsky.

Blood sera from several animal species, including the mouse, hamster, guinea-pig, monkey and rabbit, have the property, in the fresh state, of reducing the titer of certain neurotropic viruses, when the virus and serum are brought together under favorable conditions before inoculation.

The nature of this inhibitory factor is being investigated. Thus far it has been found that the inhibition is a function of temperature and length of incubation. It does not depend on the route of inoculation, and heat at 56 to 60°C may or may not destroy part of the inhibitory effect.

*12. DIAGNOSIS OF ENCEPHALITIS VIRUSES BY ANIMAL INOCULATION AND COMPLEMENT-FIXATION TESTS by Dr. J. Casals.

A great deal of the work of this laboratory consists of making such tests at the request of the Commission, the Army and Navy, and others, and preparing and distributing standard sera and antigens for the tests.

With respect to complement-fixation tests, a simple method by means of heat (60 to 62°C for 1/2 hour) for inactivation of certain antigens for safe handling in laboratories has been devised. The Japanese B, West Nile, Russian spring-summer, St. Louis, and Western equine encephalitis and louping-ill antigens can be so treated successfully, but not lymphocytic choriomeningitis or Venezuelan equine antigens. In the case of the Eastern

equine encephalitis antigen, the value of this method is dubious.

During the last six months, about 100 sera received from Army, Navy, and civilian sources, both here and abroad, have been sent to us for complement-fixation tests. All were negative with different encephalitis virus antigens except one, a Canadian serum, which was positive for lymphocytic choriomeningitis. Twenty spinal fluids and two brain specimens were received. Animal inoculation tests were negative in all instances.

About 1,000 cc. of lyophilized, inactivated antigens have been distributed, along with samples of standard antisera against different viruses, exclusively to the Army and Navy.

13. EXPERIMENTS IN INFECTIOUS HEPATITIS by Maj. W.P. Havens, Jr., M.C.

Recent experiments with infectious hepatitis carried out at the Commission's laboratory at the Yale University Medical School have dealt with the following points:

1: Determination of the period of infectivity of patients with the disease.

2: Determination of the infectivity of urine and naso-pharyngeal washings obtained in the acute phase of infectious hepatitis.

3: Determination of whether patients with infectious hepatitis induced by parenteral inoculation eliminate virus in the stool.

4: Hematologic changes appearing during the incubation period and course of infectious hepatitis in human volunteers.

5: Roentgenologic and gastroscopic findings in volunteers with infectious hepatitis.

6: Attempts to transmit infectious hepatitis to chimpanzees.

The strain of infectious hepatitis virus used in all the experiments was originally derived from the stool of a U. S. soldier who contracted epidemic hepatitis in Sicily in September 1943. This virus has been through four passages in human subjects. It is filtrable, heat resistant, and is regularly present in the stool and serum of patients in the acute,

pre-icteric phase of the disease. It has produced infectious hepatitis in 70% of human volunteers following ingestion or parenteral inoculation with incubation periods ranging from 15 to 34 days.

1. Infectivity Tests.- (A) Although acute-phase serum and stool of volunteers with experimentally induced infectious hepatitis are known to be infectious, the period of infectivity has not been previously defined. Serum and stools were obtained therefore during the incubation period, the pre-icteric phase of the disease, and in convalescence, i.e., 28 days after onset of disease, in one volunteer who had contracted infectious hepatitis 24 days after ingesting infectious serum. Serum from this patient was inoculated parenterally and his stool was fed to human volunteers. The infectivity of the acute-phase serum and stool was confirmed by positive tests in 5 of 9 human volunteers who received these materials. Neither serum obtained midway through the incubation period nor stools in the convalescent phase of this one volunteer were infectious. Dosages varying from 0.01 to 0.5 cc. of infectious serum (acute phase) caused no significant variation of incubation period.

(B) This experiment was repeated using a pool of material consisting of acute-phase serum, stools, urine, and naso-pharyngeal washings, and convalescent stools, from five patients with experimentally induced infectious hepatitis which had resulted from feeding infectious materials. The infectivity of the acute-phase serum and stools was again evident, with 5 of 6 subjects who received this material, contracting the disease. Both urine and naso-pharyngeal washings failed to produce the disease in 6 volunteers. Convalescent phase stools were again negative in four subjects.

(C) Although it is known that patients with both naturally occurring infectious hepatitis and infectious hepatitis experimentally induced by feeding infectious materials, eliminate virus in the stool, it had not been determined whether patients with experimental infectious hepatitis induced by parenteral inoculation also eliminate virus in the stool. This is of

collateral interest in attempting to relate the naturally occurring disease to homologous serum jaundice for in the latter condition, which has been produced experimentally only by parenteral inoculation (with the exception of the single transmission to a human subject effected by MacCallum and Bauer by feeding the icterogenic agent), virus has never been recovered from the stool. Acute-phase serum and stool were pooled from 8 volunteers whose disease had been induced by parenteral inoculation of our strain of infectious hepatitis virus. All of these men had had short incubation periods ranging from 20 to 30 days. Virus was eliminated in the stools of such patients during the acute phase of the disease, producing infectious hepatitis in 3 out of 3 volunteers after ingestion. It was also present in the acute-phase serum, producing the disease in 2 out of 3 volunteers.

2. Hematology.- It has been pointed out for several years by American and British workers that atypical mononuclear cells appear in the peripheral blood during the course of infectious hepatitis.

Systematic leukocyte counts on our experimental infectious hepatitis subjects have revealed a regular pattern of change, characterized by the early appearance of leukopenia with both absolute and relative lymphopenia and relative polymorphonuclear leukocytosis. Subsequently the neutrophils are depressed to such a low level that a relative lymphocytosis exists. Although minor changes may occur 2-3 days before the onset of fever, the striking changes are primarily associated with fever. Those patients whose onset is acute with fever, manifest the hematologic changes within the first 24 to 36 hours of disease while those whose onset is gradual with vague generalized or abdominal complaints, have the characteristic blood changes several days later coincident with the beginning of fever. The degree of change in the blood studies apparently parallels the severity of the febrile response.

In all but one patient, leukopenia, i.e., a reduction to below 5000 leukocytes/cu.mm., was found at some time during the pre-icteric acute phase of the disease.

Of interest has been the appearance of the lymphocytes during the acute phase. As early as the first or second day when lymphopenia is pronounced an occasional atypical lymphocyte is seen. Such cells are characterized by their large size and characteristic changes in the nucleus and cytoplasm. They resemble cells seen in infectious mononucleosis. These cells increase in percentage and absolute numbers until the fifth to seventh day of fever when lymphocytosis is present. At this time they may number as many as 18 to 20 per cent of the total lymphocyte count. By the tenth day when the lymphocyte count has assumed normal proportions of the total count, these atypical lymphocytes have largely disappeared. Their appearance is correlated in many patients with the appearance of posterior cervical adenitis.

3. *Gastroscopy*.- (with Dr. S. Kushlan and Dr. R. Green) Gastroscopic examination of patients with infectious hepatitis by Knight and Cogswell showed changes from normal in the appearance of the gastric mucosa in 7 out of 9 patients during early pre-icteric and icteric phases. The findings were described as those generally interpreted as superficial gastritis involving primarily the antral portions of the stomach and in some instances the fundus. In five of the patients small aphthous ulcers from two to five millimeters in diameter were reported in the antrum and on the angularis.

In repeating this work on our experimentally induced cases we have had an opportunity to study the appearance of the gastric mucosa before, during, and after the course of the disease. Six men had gastroscopic examinations before inoculation, during illness, and after recovery (2 months after onset) from infectious hepatitis. During the disease gastroscopy was performed from the third to the seventeenth day of illness.

Of the six patients who were gastroscoped before, during, and after experimentally induced infectious hepatitis, two showed unequivocal changes. These two (SN and TR) revealed perfectly normal mucosa in the antrum and body of the stomach, both before infection and after complete subsidence of the infection. The changes during the acute phase of disease were the same

in both patients, differing only in degree, and consisted of a marked crimson red appearance with edema, both more acute in SN who was examined earlier in the illness (pre-icteric) than in TR (7th day of jaundice).

In another man (VW), the changes seen were suggestive. Prior to infection, the gastric mucosa appeared normal. During his illness, the mucosa was fiery red and edematous as in the last two. However, on the final post-illness control examination, similar changes were noted but the redness was less fiery, bordering on an orange-red; edema was less marked. This would seem to represent a subsiding superficial gastritis. In the other 3 cases, the changes were entirely equivocal and from a gastroscopic viewpoint were inconclusive.

4. Roentgenograms.- X-rays of the gastro-intestinal tract after an opaque meal, were made in 12 subjects before inoculation and from 10 to 17 days after onset of infectious hepatitis. It was concluded that no definite constant pathology was determined by radiological examination of these 12 subjects examined before inoculation and during the acute phase of infectious hepatitis.

5. Attempts to infect 6 chimpanzees (2 adults, 4 young) with proven infectious hepatitis virus were unsuccessful.

14. SERUM JAUNDICE by Dr. C. A. Janeway.

1. Frequency in a Civilian Hospital.- Observations on the frequency of jaundice following the transfusion of blood and plasma procured and issued through the blood bank of a Boston hospital indicate that this disease occurs more frequently in this country than is usually realized. The usual plasma pool in this blood bank is made up from an average of 16 bleedings. One plasma pool (#219) is of particular interest. Of the seven recipients who survived long enough to develop jaundice, four (all adults) developed hepatitis approximately 90 days after transfusion and one died. The fifth and sixth recipients, aged 7 and 19 months escaped. All the donors to the pool have

been traced and questioned by interview if possible or else by mail; except two, who are in the Navy and are now being traced. Of the 13 donors questioned, two have interesting histories. One is known to have congenital hemolytic jaundice and had a splenectomy. Her child was having a splenectomy for that condition at the time of her donation. The other has a much more suspicious history. Four years before his donation, he and his wife had "intestinal grippe with a touch of Jaundice." Although the donor has been well since, his wife has had recurrences of intestinal grippe with slight jaundice every winter since then.

2. Serum Jaundice in Relation to Plasma Fractionation.- Two features of the process of plasma fractionation would appear to favor the dissemination of hepatitis virus in its products: First, the large size of the plasma pools (250-10,000 bleedings), and second, the gentleness of the fractionation procedure which is carried out using alcohol as a precipitant at temperatures below freezing point of water. Studies by Drs. W. C. Boyd and J. F. Enders showed that two viruses (Theiler's mouse encephalomyelitis virus and tobacco mosaic virus) added to the starting plasma could be detected in all the major fractions. For this reason an attempt has been made to follow as many patients as possible who have received albumin and gamma-globulin. The results indicate that up to the present these two products have probably not transmitted hepatitis to any recipients.

3. Thermal Death Time of Agent of Serum Jaundice. Because the possibility of transmission of hepatitis by human serum albumin had not been eliminated, it was decided to determine whether the virus could be killed by heat in the presence of albumin. An experiment has been carried out by Dr. George Scatchard, Dr. L. E. Strong, and myself, working in collaboration with Dr. Joseph Stokes and Capt. Sidney Gellis of the Commission on Measles and Mumps. On the basis of Dr. J. Murray Luck's discovery that nonpolar anions would increase the thermal stability of albumin, it has been possible to develop a low salt concentrated albumin solution, stabilized with either .04 M

acetyl tryptophanate or a mixture of .02 M acetyl tryptophanate and .02 M caprylate at a pH of $6.8^{-0.2}$. The first solution can be heated to a temperature of 60°C for approximately 18 to 20 hours, the latter at 64°C for approximately the same period, before visible turbidity develops.

A sample of Fort Bragg plasma (derived from cases of yellow fever vaccine jaundice) was added in a proportion of 1 volume of plasma to 4 volumes of concentrated albumin solution. Three aliquots of this mixture were treated as follows:

A - (Albumin with .04 M acetyl tryptophanate) held in the cold room, for control.

B - (Albumin with .04 M acetyl tryptophanate) heated 10 hours at 60°C in a water bath.

C - (Albumin with .02 M acetyl tryptophanate and .02 M caprylate) heated 10 hours at 64°C in a water bath.

At the end of the heating period, the three mixtures were frozen at 70°C, packed in dry ice and shipped to Capt. Gellis, who administered them by intramuscular injection to a group of volunteers the following day. Each man received an inoculum of 10 cc. containing 2 cc. of plasma, except the controls who received approximately half this dose.

Results: Although the experiment is not completed (15 weeks have elapsed), so far five out of ten controls have developed hepatitis, while, of the five men in each group receiving mixtures B and C, none have developed any evidence of hepatitis.

15. INFECTIOUS HEPATITIS. LESIONS IN LIVERS OF NORMAL MICE by Drs. P. K. Olitsky and J. Casals.

In normal, Rockefeller Institute or Swiss strains of albino mice, certain spontaneous lesions were observed which should be considered in connection with attempts to transmit liver affections to this animal. One type consists of infiltration about the bile ducts, also about blood vessels, of polymorphonuclear (predominantly) along with monocytic, lymphocytic and

plasma cells. Associated is a focal necrosis of individual hepatic cells. Another type is characterized by the presence of intranuclear inclusion bodies. The relation of one type of lesion to the other is as yet undetermined; the inclusion bodies are found only in older mice and the infiltrative lesion is noted especially with increasing age. There is some resemblance between the picture of the inclusions to those reported by Findlay only in a particular (Clacton) strain of mice; their identity is a matter for further study.

Liver Function Test in Mice.- Recently Maclagen described a new test for liver function in man, the thymol turbidity test. In connection with the spontaneous lesions found in mice, as described above, we undertook an investigation of the value of this test applied to normal mice and to mice having pathological conditions in the liver spontaneously developed or artificially induced. Thus far a normal base line for this animal has been established.

In the discussion Capt. Schlesinger reported spontaneous jaundice occurring in 1 mouse whose serum bilirubin was 20 mg. per cent. Sections showed necrosis of liver cells. Passage to mice and other animals was unsuccessful.

16. POLIOMYELITIS IN 1945. THE OUTBREAK AT FORT McCLELLAN, ALABAMA by Dr. R. Ward.

An unusual, sharp and moderately serious outbreak of 17 cases of poliomyelitis occurred at Fort McClellan, Alabama, during March and April 1945. Among these 17 cases there were 10 with bulbar symptoms; 3 of these were fatal. An investigation was carried out to determine if possible, what factors were involved in the dissemination of virus through this camp. The cases were grouped rather closely in point of time of onset, but scattered throughout the camp from a geographical standpoint. Their chronological grouping suggested that a large amount of virus was rather suddenly introduced

by unknown channels into the camp population. The spotty distribution of cases throughout the camp is compatible with the theory that there may have been a very wide dissemination of virus to which many could have been exposed but only an occasional susceptible acquired the disease among this fairly resistant population of adults. The onset of the epidemic was heralded by a single diagnosed case which preceded the others by 3 or more weeks. He and/or other undiagnosed cases or carriers, might have been _entirely_ responsible for spreading the virus throughout the camp. We believe this to be unlikely.

It is pertinent to call attention to the fact that a soldier at the nearby but much smaller Camp Sibert (about 40 miles distant) also contracted poliomyelitis during the period of the epidemic at Fort McClellan. The Camp Sibert patient had not visited Fort McClellan. Mention of this fact is made to indicate that intrinsic factors of spread at Fort McClellan may not have been entirely responsible for the prevalence of poliomyelitis in this fairly small area of two counties. Three civilian cases were also recorded in nearby counties in the month preceding the camp epidemic.

The investigation has been conducted with the idea that the manner of spread of poliomyelitis in _any_ epidemic is unknown. A number of different modes of spread of poliomyelitis virus deserve consideration in the Fort McClellan outbreak and at Camp Sibert. Infection might have been entirely the result of exposure to infective human carriers, or to contaminated food, or milk or ice cream, as well as to some other extra-human source of infection. Contamination of water supply seems unlikely. Tests carried out on water, milk, ice cream, sludge and flies were negative for poliomyelitis virus.

Although no definite answer can be made as to the manner of spread in this outbreak, it is pointed out that epidemics of poliomyelitis in Army camps, occurring as they do in a population which is more controlled than in civilian populations of the same size, offer certain opportunities which should be of considerable value in tracing the spread of this disease if enough epidemics of this type are adequately studied.

17. CIVILIAN EPIDEMICS OF POLIOMYELITIS IN THE EAST - 1945 by Dr. J.R. Paul.

Individual epidemics were not enumerated but a few items seen during the past summer were mentioned.

(1) The occurrence in July 1945 during the epidemic at Paterson, N.J., of a severe flood within the epidemic urban area during the period in which the epidemic was getting under way. The flood inundated about 1/10 of the city and caused sewage to be floated into many city streets at a time when there must have been many intestinal carriers of virus in the city. The subsequent course of the epidemic did not seem to be influenced in any particular direction by this flood.

(2) Attention was called to the high prevalence of bulbar cases and the high mortality (similar to the epidemic at Fort McClellan) which was noted in the epidemic at Rockford, Ill., in July and August 1945.

(3) Mention was made of the death of a technician working with recently isolated strains of virus in the Yale Poliomyelitis Study Unit. The fact that two such laboratory accidents have now occurred, would indicate that exposure to "human" poliomyelitis virus in the laboratory is probably more dangerous than has been originally suspected.

(4) Mention was made of a family of 8 children seen in August 1945 at Rockford, Ill.,in which 8 cases of illness occurred including: 1 death, 3 paralytic cases and 4 abortive or questionable cases. Events which transpired in this family offered an unusual opportunity for contrasting the early with late symptoms of the clinical disease.

18. ENCEPHALITIS AND POLIOMYELITIS IN THE WEST by Dr. W. McD. Hammon.

A. Kern County, California, 1944, Final Report.- The list of apparent neurotropic virus infections seen at the Kern County General Hospital was finally reduced to 57. These can be divided now into 5 groups: Group I, in 17 cases because of unsatisfactory specimens no etiologic diagnosis could be made. Group II, 4 cases of St. Louis (St. L.) encephalitis were diagnosed

by showing an increase in the antibody titer. Group III, 2 cases of Western equine (W.E.) type encephalitis were diagnosed by demonstrating an increase in titer. Group IV, 4 patients had flaccid paralysis; their feces were tested in monkeys with negative results. Group V, the 34 remaining patients, although many of them had antibodies to either the St. L. or the W.E. virus, were shown to have no increase in antibody titer to either virus during the period of the illness. Feces from 16 out of the 34 patients were tested in monkeys for poliomyelitis virus, but none was isolated. In view of the failure to detect poliomyelitis virus by methods which were successful with feces from the same region the previous year and from other regions during the same year, we do not feel that the 38 cases in the last 2 groups necessarily represent poliomyelitis.

B. The California Virus.- Immunological tests show quite conclusively that the California virus is not similar to or closely related to Theiler's (TO) or Theiler's GD-VII viruses, nor to the 3 Columbian viruses, Anopheles A, Anopheles B, or Wyeomyia.

Results of neutralization tests suggested that ground squirrels and rabbits might be the reservoirs of this virus in Kern County. These animals therefore were tested to determine whether virus could be found circulating in the blood following peripheral inoculation. It will be recalled that negative results had been obtained with mice, hamsters, guinea pigs and cotton rats. Virus was detected quite regularly in the blood of both the ground squirrel and the rabbit following peripheral inoculation of a small amount of virus; then within 30 days a relatively high titer of neutralizing antibody could be detected in the blood of these animals. A single small scale attempt to transmit this virus to the rabbit by Aedes dorsalis has been unsuccessful.

C. Mosquito Transmission of Encephalitis Viruses.- Aedes dorsalis have now been shown capable of transmitting the St. L. virus in the laboratory. Since we found this mosquito infected naturally with the St. L. virus in California in 1944, it must be considered as a potential vector.

A satisfactory experiment with Culex tarsalis with Jap B virus has finally been completed. The mosquitoes were found capable of transmitting the virus to mice. This makes the seventh California mosquito found capabl of transmitting this virus.

D. Japanese B Virus in Chick Embryos.- The Jap B virus has been carried in chick embryos for over 50 passages by Dr. Robert B. Lawson. Titrations of the 50th passage gave the following results: Dilutions of the whole embryo and of the amniotic sac to 10^{-8} killed all mice in all dilutions. These results are at least 1 or 1 1/2 logs higher than those obtained with the 39th and earlier passages. The chorioallantoic membrane killed all mice through the 10^{-6} dilution as did also the yolk sac. The LD-50 for the amniotic fluid and the allantoic fluid fell between 10^{-6} and 10^{-7}. Nine day embryos were used, and they were inoculated in or near the amniotic sac. Embryos died 40 to 46 hours after inoculation and all were deeply hemorrhagic. We conclude that a definite adaptation has finally occurred. This confirms the work reported by Warren.

E. Complement Fixation Test With Japanese B Encephalitis.- Experimental complement fixation antigens have been prepared with various tissues of the chick embryo, beginning with the 45th passage. Up to the present time considerable irregularity has been noted in titers of the antigens from one lot to another, and it is apparent that the mouse infectivity titer does not necessarily parallel the complement fixing titer. The whole embryo has shown the highest titer as an antigen in most instances and although results are occasionally good with the chorioallantoic membrane or the amniotic sac, they are by no means regular. Titers of human sera from Okinawa have exceeded 1:64 and rabbit and hamster immune sera have shown titers up to 1:128.

F. Complement Fixation Tests for the Western Equine Virus.- Chick embryo antigens for the W.E. complement fixation test have been prepared by Mr. Carlos España. Although the chick embryo infected with the California strain of W.E. virus is a relatively satisfactory antigen as far as titer is

concerned, a fairly large number of normal human sera give positive results with normal chick embryo suspensions. Since in addition, other tissues actually give higher titers and appear to be more specific, the use of the whole embryo has been abandoned. After 19 passages in the embryo, almost any tissue has made a fair antigen; after 30 passages the membranes have been found to be the most satisfactory antigens. In most instances they are used in a 2.5 per cent dilution after making a preliminary antigen titration.

Approximately 200 human sera from Kern County patients of 1945 have been tested up to the present time. Convalescent titers have reached 1:256 (4+) and 1:512 (2+). None of the sera tested has given a positive reaction with control normal chick embryo membranes and fluid, and none of a relatively large group tested against the same tissues infected with Japanese B has given a positive reaction.

Titrations of neutralizing antibodies are being made on all these sera. In general, there has been good correlation between a positive C.F. test and a positive neutralization test.

G. Japanese B Virus in Chickens.- In order to explore the possibility of birds as reservoirs of the Jap B virus, chickens were inoculated subcutaneously with minute doses of virus (.3 cc. of a 6 LD-50 mouse brain) and the sera were tested at 12 hour intervals for the presence of circulating virus. Bleeding was begun at 12 hours and the final bleeding taken at 192 hours. Virus was first isolated at 48 hours, again at 72, 84, 96, 144 and 168 hours. Spleens were removed and tested for virus at 120, 144, 168 and 192 hours. In no spleen was virus found to be present. Unfortunately, no recently isolated strain of virus was available for these experiments. Since they were performed with a brain-adapted strain, we do not feel that the results are indicative of what occurs in nature. Under the conditions of this experiment, virus was found quite irregularly in the blood of any one chicken and at no time was it detectable in a dilution of serum greater than 1:10.

H. Investigation of "Encephalitis" in the Navy Training Station in San Diego, California, Spring of 1945.- During the spring of 1945 before the onset of the season for encephalitis and poliomyelitis, a request was received from the Navy to investigate a series of cases diagnosed as "encephalitis" which occurred at the Naval Training Station at San Diego. We concluded that these were probably not cases of any epidemic virus encephalitis or of poliomyelitis, nor did there seem to be any particular epidemiological relationship between the cases. In one case, considered clinically as a possible mumps meningo-encephalitis without parotitis, laboratory studies later confirmed this diagnosis. Neutralization tests against the W.E., St.L. and Jap B viruses were negative.

I. Poliomyelitis Epidemic in Mill Valley, California.- An epidemic of poliomyelitis occurred this summer in Marin County, just across the Golden Gate from San Francisco. Most of the cases were in and around a small town of about 6,000 population (Mill Valley). The onset of the first case was on June 9th; 16 to 18 days later there were several more cases and the final total of probable cases was 30. An epidemiologic investigation was begun at the time of the second case. Somewhat later, on June 27th, we learned of 2 cases in a trailer camp of approximately 200 trailers (government owned and operated) near Mill Valley. Both had their onset one day before our first visit. A hasty survey indicated that this isolated trailer community would serve as an excellent place to conduct an intensive epidemiological and laboratory study of poliomyelitis. The population was mixed Army, Navy and shipyard workers. Daily clinics at the camp were organized immediately with the assistance of Dr. Lawson. Most of the children attended these regularly as did many of the adults. Temperatures were taken, questions as to symptoms were asked, and anyone who was ill was carefully examined for any signs of poliomyelitis. Every person attending had his throat swabbed to obtain a specimen for virus tests. Throat swabs were collected every other day and if there were any suspicious signs or symptoms daily swabs were taken. Fecal specimens were also collected from any suspicious person.

Seven more cases of poliomyelitis occurred in the camp during the period of investigation, making a total of 9. In addition there were approximately 50 mild illnesses which might well have been poliomyelitis. Measles and mumps were present in the trailer camp at the same time; the onset of the first cases of each of these diseases occurred approximately at the same time as the first cases of poliomyelitis. We thus had an opportunity to compare the spread of 3 communicable diseases in the same community simultaneously. In all there were 19 cases of measles, 4 of mumps, and 9 of poliomyelitis.

A survey of the trailer camp revealed good sanitary facilities in general. The kitchen sinks of the trailers, however, emptied into a sluggish creek which ran through the camp, dividing it in half. The creek also received cow barn washings from a nearby farm just before reaching the camp. Flies and fly breeding were abundant in creek and cowbarn. Their numbers were reduced after the Health Department sprayed DDT in the creek and cowbarn. All of the cases of poliomyelitis except the last one occurred on the west side of the creek. Mumps began on the east side and spread to the west late, whereas the reverse was true for measles.

The testing of throat swabs which is being carried out by Mr. Walter Mack, is as yet incomplete. So far 3 specimens have been positive: one from an adult patient 24 hours after onset; one from a 1 1/2 year old infant who had fever (105°F) without other symptoms; and one from this infant's father who at the time of collection of the specimens, complained of vague symptoms; this was 8 days before his admission to a hospital with a diagnosis of poliomyelitis. Unfortunately, much of the other material has been tested in animals with tuberculosis. The clinical and histological pictures have been difficult to interpret.

Mouse neutralization tests using the Lansing strain have indicated a definite rise in titer in 5 of 13 patients' sera. The other 8 showed no change.

An unusual feature of this outbreak is the age distribution. Over 50

per cent of the cases in the trailer camp were among adults and an unusually high proportion of adults were involved in the county-wide outbreak.

J. Encephalitis and Poliomyelitis - Kern County - 1945.- This has been a year of epidemic proportions for encephalitis in horses and man, and certain areas have had poliomyelitis rates well above normal. So far one W.E. virus has been isolated from a fatal Kern County patient, and 17 have been diagnosed as due to the ~~E~~W.E. virus by serological tests (principally C.F. test). A large proportion have antibodies to W.E. or St.L. or both on convalescent specimens but as usual these probably are for the most part evidence of a previous undiagnosed infection. Two cases of mumps meningo-encephalitis without parotitis were diagnosed by the complement fixation test. Several other cases were similarly diagnosed from San Francisco hospitals, all previously diagnosed as poliomyelitis.

Mosquito collections have all been part of the DDT control experiment reported separately. A total of 8,478 Culex tarsalis have been tested to date, and 27 viruses isolated. Twelve have been identified as W.E. virus, one as St.L. and identification is incomplete for 14 strains.

K. DDT Encephalitis Control Program.- The first phase of the DDT control program which is being carried out by Dr. Reeves and his assistants, and outlined in a previous report, has now been terminated. In DDT-sprayed chicken houses and control area chicken houses 2,479 mosquitoes were collected of which 340 were Culex tarsalis. From other buildings in the 2 areas 16,071 mosquitoes were collected, of which 4,865 were C. tarsalis. From chicken houses in a zone two miles outside the experimental areas, where no special control measures were used, 13,611 mosquitoes were collected, 7,556 of which were C. tarsalis. DDT exercised a definite affect on mosquitoes on the areas sprayed but did not eliminate Culex. C. tarsalis appeared to be affected more than Culex quinquefasciatus, the other common species which persisted. Anopheles were rarely encountered.

The following facts not previously known became apparent: 1) DDT in Diesel oil on unpainted, dry wood is carried deep below the surface leaving almost no detectable residual after a very short period of time. Within a month after the first spraying it was found necessary to respray, instead of after three months as planned. In xylene-water emulsion a better result was obtained on these surfaces. 2) Any single area left unsprayed (upper surface of board on rafters, between shingles, inside of box) or the unsprayed surface of any article introduced after spraying could usually be found harboring mosquitoes in fairly large numbers. It is claimed that with Anopheles reasonable coverage will eliminate them. 3) Intensive larval control measures plus residual DDT spraying of most buildings and known favorite resting places for mosquitoes about domestic buildings, as performed in our area B, has only a moderate effect on reducing the population of Culex mosquitoes.

To our surprise we learned that infection rates of C. tarsalis in the sprayed chicken coops was higher than in the unsprayed coops outside either control area, 1 per 108 and 1 per 674 respectively (average for both areas). Small numbers may explain the findings for only 3 and 5 viruses were isolated, from 324 and 3,371 tested mosquitoes, respectively. Isolations from shelters other than chicken coops within the two areas gave an infection rate of 1:251 (average for two areas).

Although the infection rate for Area A (DDT in chicken houses and no larval control) is 1:212 and that for Area B (Larval control with DDT residal more extensively) is 1:364 there is probably no significant difference. Moreover it cannot be said with certainty that the infection rates of the two areas (15 miles apart) would have been equal without any control measures. The only obvious conclusion that can be drawn from the experiment is that inexpensive or moderately expensive mosquito control (as carried out in Areas A and B respectively) and even relatively intensive larval control measures including the use of DDT do not eliminate or greatly decrease infection rates

in the remaining mosquitoes (of which there appear to be plenty). Neither does the former method eliminate human and horse infections for one of each occurred in the center of Area A.

Several hundred sera are now being collected from chickens and wild birds and mammals in each experimental area and from their surrounding control zones. These will be tested to determine infection rates for comparative purposes. Final conclusions regarding the effectiveness of the control procedures must await completion of these studies.

19. MURINE POLIOMYELITIS VIRUS IN THE CHICK EMBRYO by Mr. J. T. Riordan.

In 1943 Gard reported the transfer of Theiler's FA mouse poliomyelitis virus to the developing chick embryo and propagation through four passages. Using the chorioallantoic technique, Gard demonstrated the presence of virus only in the central nervous system (CNS) of embryos which had been inoculated at the age of 5, 6 and 7 days. In eggs inoculated at 8 days of age, no virus was recovered from any site. In no instance was virus discovered in membranes or in the embryo minus the brain and vertebral column. Also, CNS harvested 10 days after inoculation was infective for mice inoculated intracerebrally but CNS harvested 5 days after inoculation was not infective when tested in the same manner. In Gard's work, eggs were incubated at 37°C before, as well as after, inoculation. The inoculum consisted of 0.05 ml. of a 10 per cent suspension of infected mouse brain. Gard also claimed one successful passage of the UFI strain (fecal origin) of mouse poliomyelitis to eggs.

In our work with murine poliomyelitis in fertile eggs, two strains have been passed through five generations: one Theiler's FA and the other a strain recently adapted to rodents in this laboratory. Allantoic fluid, amniotic fluid, chorioallantoic membrane, chick embryo brain and embryo minus the CNS, harvested both 5 and 10 days after inoculation, proved infective for mice inoculated intracerebrally. The highest titre appeared to be in the embryo, harvested 10 days after inoculation.

Our results differ with those reported by Gard both as to the incubation period and neurotropic properties of FA virus in fertile eggs. However, a lower incubation temperature (35-35.5°C) and a larger inoculum (0.1 ml.) was used in our experiments.

20. <u>FLY ABATEMENT STUDIES IN URBAN EPIDEMICS OF POLIOMYELITIS</u> by Drs. J. L. Melnick and R. Ward.

These experiments were designed to answer 2 questions:

1) Could the fly population within cities be reduced by D.D.T.?

2) Could a reduction in the number of flies, if achieved, be correlated with the incidence of poliomyelitis?

It seemed to us that this approach might give a direct answer as to whether or not flies play a part in the transmission of poliomyelitis.

D.D.T. was applied in 2 urban epidemic areas, each comprising about 4 square miles and inhabited by 67,000 and 28,000 people respectively. Although a temporary (5 to 20 days) reduction in flies was achieved in both, too few cases of poliomyelitis developed in either sprayed area or its control subsequent to the spraying to indicate that the spraying altered the epidemic.

Thus, under the circumstances which were not ideal, there was no effect on the poliomyelitis epidemic in either area.

The meeting adjourned at 4:15 P.M.

A moving picture film taken by the Signal Corps on Okinawa was shown subsequently.

Respectfully submitted,

John R. Paul, M.D.
Director

30 October 1945

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