nephritis may in fact be more closely related to water and salt retention than to renin secretion.

The relationship of aldosterone to sodium balance in response to the formation of renin or angiotensin has been discussed. The administration of renin causes an increase in the width of the zona glomerulosa layer of the adrenal gland, and this is one of the bits of evidence in favour of this site of formation of aldosterone. Conn's syndrome of primary aldosteronism is usually associated with moderate hypertension, which is reduced by removal of the tumour: the associated potassium loss can also induce tubular damage and pyelonephritis (Milne *et al.*, 1957) with other consequences on the blood-pressure. The obviously treatable hypertension of phaeochromocytoma we can regard as a partially solved problem. The careful work of von Euler (1956) has shown quite clearly that in the vast majority of essential hypertensives there is no excess excretion of noradrenaline. The distribution of vasoconstriction under the influence of noradrenaline is also entirely different from that seen in essential hypertension or induced by angiotensin. While cortisone by itself may not induce hypertension, in Cushing's syndrome some other unidentified intermediary steroid may be responsible (Grob, 1960): pressure often subsides slowly over a few months after adrenalectomy.

[The second lecture, together with a list of references, will appear in our next issue.]

VACCINATION AGAINST MEASLES

PART I. PREPARATION AND TESTING OF VACCINES CONSISTING OF LIVING ATTENUATED VIRUS

BY

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In 1958 Katz, Medearis, and Enders showed that the Edmonston strain of measles virus adapted to chick-embryo-tissue cultures was considerably attenuated for both monkeys and man. Live virus vaccines for trials in the United States were prepared by them (Enders *et al.*, 1960) and by other workers from the same strain (Stokes *et al.*, 1960; Schwarz *et al.*, 1960).

The studies reported here were undertaken to determine the degree of attenuation shown by four batches of measles vaccine derived from different sublines of the Edmonston strain. Two of these batches were prepared at these laboratories and the other two in the United States. The preparation and testing of the vaccines is described in this paper; Parts II and III describe the clinical and antibody responses of children in clinical trials in England and Nigeria.

Materials and Methods

Strains of Virus.—The passage history of the four vaccine strains is summarized in Table I. The early passages of all four were done by Enders *et al.* (1960). The 3C subline was started from the fourteenth-chick-embryo-tissue-culture passage received from Dr. S. L. Katz. The fifteenth to thirty-first chick-tissue-culture passages were made in these laboratories. From the eighteenth passage onwards the incubation temperature was lowered to 32° C. Passages 27 and 28 were made at limit dilution. The 4A subline corresponds closely to vaccine B of Enders *et al.*; the final three chick-tissue-culture passages were made in these laboratories, starting from material received from Dr. S. L. Katz. The subline for vaccine 8 had been passaged 39 times in the Parke, Davis Laboratories after receipt from Dr. S. L. Katz. Vaccine L was prepared for Dr. S. L. Katz in the Merck Sharp and Dohme Laboratories, five passages beyond the vaccine A of Enders *et al.*

TABLE I.—*Passage History of Vaccine Strains*

Early Passages	Vaccine	Additional Passages
Edmonston strain: 24 in human kidney + 28 in human amnion + 6 in embryonated eggs	3C	17 in chick-embryo-tissue cultures at 37° C. 14 ,, ,, ,, ,, ,, ,, 32° C.
	4A	13 in chick-embryo-tissue cultures 6 in embryonated eggs 1 in chick-embryo-tissue cultures at 37° C. 3 ,, ,, ,, ,, ,, ,, 32° C.
	8	53 in chick-embryo-tissue cultures
	L	19 in chick-embryo-tissue cultures

Titrations of Virus.—Serial dilutions in 0.5 log steps were prepared and 0.5-ml. volumes were inoculated into HEp-2 cell cultures, using five cultures per dilution. Cultures were incubated in a roller apparatus at 37° C. and were examined microscopically for the cytopathic effect of measles virus at intervals up to 14 days after inoculation. Titres were calculated by the method of Kärber and were expressed as TCID50 per 0.5 ml.

Titration of Neutralizing Antibody.—The sera from experimental animals and from vaccinated children were examined for neutralizing antibody. Pre- and post-vaccination samples were examined as a pair in the same test. The sera were inactivated at 56° C. for 30 minutes and diluted, using twofold dilution steps. Equal volumes of serum dilution and virus at a final dilution of 100 TCD50 per 0.5 ml. were incubated at 20° C. for one hour. Two or four HEp-2 cell cultures were each inoculated with 0.5 ml. of the mixture. Cultures were observed for 14 days for cytopathic effect. The neutralization end-point was calculated by the method of Kärber. Titres are expressed as the reciprocal of the final dilution in the serum–virus mixture.

Preparation of Vaccines

Vaccines 3C and 4A.—Virus was grown in chick-embryo-tissue cultures. Primary and secondary seed lots were produced under the same conditions as vaccine lots. Trypsinized cells from decapitated chick embryos of 7–11 days' incubation were grown at 37° C. in Roux flasks, using Earle's balanced salt solution plus 0.5% lactalbumin hydrolysate, 5% of heated normal horse serum, and neomycin and polymyxin as antibiotics. Confluent cultures were washed twice with Earle's balanced salt solution, the seed virus inoculum was introduced and left in contact with the cell sheet for one hour at room temperature. At the end of this period the cell sheet was washed twice with Earle's balanced salt solution and re-fed with synthetic medium 199. Inoculated cultures were incubated at 32° C. for

8–12 days, without change of medium. Periodically cultures were inspected and those showing signs of toxicity or evidence of bacterial or fungal contamination were discarded. At the end of the period of incubation the cultures were subjected to one cycle of freezing and thawing, gross cell debris was removed by filtration through gauze, and the filtrate from all the cultures was pooled. Secondary seed lots were distributed in convenient amounts into glass containers and stored frozen in a CO_2 cabinet. Bulk virus pools were stored at either $+4°$ C. or $-70°$ C. until processed into final vaccine. A stabilizer was added to the bulk virus, which was then filled in 0.5-ml. amounts into glass ampoules and freeze-dried. The final dried vaccine was stored at $-20°$ C. For use an ampoule was reconstituted with 0.5 ml. or 1 ml. of sterile distilled water.

Vaccine 8 was prepared from liquid vaccine batch No. B 091265 sent to us by the courtesy of Parke, Davis and Co. Ltd., Detroit, U.S.A. Final vaccine was freeze-dried at these laboratories, in 0.5-ml. amounts in the same way as batches 3C and 4A.

Vaccine L, which was brought to Nigeria by Dr. S. L. Katz, had been prepared as described by Enders *et al.* (1960), and was not freeze-dried. The virus was stored in flame-sealed ampoules in a dry-ice refrigerator after preparation and transported in dry ice.

Testing of Vaccines

Vaccines 3C and 4A.—Each batch of vaccine and the seed from which it was prepared was tested for potency, for attenuation for monkeys of the measles virus, and for extraneous agents of bacterial, fungal, or viral nature. Some tests used samples of bulk vaccine, while others were done on final vaccine reconstituted from dried ampoules. Tests for potency were made with the final vaccine, which was titrated for live virus content in HEp-2 cell cultures. Titrations were also done on ampoules stored at various temperatures and returned unopened from field trials. Each vaccine was also tested for antibody production in guinea-pigs. Guinea-pigs received three injections of either undiluted or 1:10 dilution of final vaccine given at weekly intervals. Three animals were used on each dilution. Serum collected seven days after the last injection was titrated for measles neutralizing antibody.

The test for attenuation was done on a sample of bulk vaccine. Three cynomolgus monkeys previously shown to be free from measles neutralizing antibodies were inoculated with each batch of vaccine, two receiving 0.5 ml. each intracerebrally and one receiving 20 ml. intraperitoneally. Temperatures were taken daily for 14 days; blood samples were collected daily for viraemia studies from the third day to the fourteenth day after inoculation, and also on the day of inoculation and the twenty-first day for serum antibody titrations.

In addition to standard procedures to exclude bacterial, fungal, and mycoplasmal (PPLO) contaminants a wide range of tests for extraneous viruses was carried out. Suckling and adult mice, rabbits, and guinea-pigs received vaccine samples by various routes. The identity of the virus was confirmed by neutralization tests in tissue culture using immune rabbit measles sera and a pair of human acute and convalescent measles sera. In addition, cultures of both monkey kidney and HEp-2 cells were inoculated with 50-ml. volumes of each vaccine neutralized with measles hyperimmune serum.

Vaccine 8.—Prior to its receipt by us, tests for bacterial sterility, for potency by live virus titration and antibody production in guinea-pigs, for extraneous agents by inoculation of mice, guinea-pigs, and embryonated eggs, and for identity by neutralization with immune serum, had been performed on this batch at the Parke, Davis Laboratories. Dr. W. W. MacLean also informed us that more than 200 children had been vaccinated with this batch without any serious effect. After freeze-drying at these laboratories further tests were performed for potency, bacterial sterility, and toxicity.

Vaccine L.—We are indebted to Dr. S. L. Katz for the following information. Safety-testing had been performed in monkeys, suckling and adult mice, embryonated hen's eggs and baby chicks, and a variety of human and animal cell cultures and microbial media.

Results

Potency.—The live virus content of the four vaccines ranged from $10^{2.7}$ TCD50 per human dose for vaccine L to $10^{3.4}$ for vaccine 4A. All the batches tested by guinea-pig inoculation produced antibody, even when diluted 1:10 (Table II).

TABLE II.—*Results of Potency Tests on Measles Vaccines*

Vaccine	Live Virus Content	Antibody Production in Guinea-pigs	
		Undiluted Vaccine	1 : 10 Vaccine
3C	$10^{2.9}$	72*	7
4A	$10^{3.4}$	161	8
8	$10^{2.9}$	5†	Not tested
L	$10^{2.7}$‡	Not tested	Not tested

* Geometric mean titre of three guinea-pigs.
† Results from the Parke, Davis Laboratories.
‡ Results from Dr. S. L. Katz.

Attenuation for Monkeys.—Some difficulty was experienced in these tests. All three monkeys injected with final vaccine 3C and one out of three injected with 4A had acquired measles neutralizing antibodies between the time when they were selected as suitable for use and the time of inoculation. Tests on the secondary seed lots proceeded satisfactorily and were regarded as valid tests of attenuation for the batches. One intracerebrally and one intraperitoneally inoculated monkey on the final vaccine test of batch 4A showed temperatures above 104° F. (40° C.) for three days and six days respectively. One intraperitoneally inoculated monkey on the secondary seed test of batch 3C had a temperature above 104° F. (40° C.) for five days. One intracerebrally inoculated monkey on the test of secondary seed of batch 3C showed viraemia from the seventh to the eleventh day after inoculation. No signs of illness were observed in these or any of the test monkeys.

Extraneous Agents.—No evidence of any extraneous viral or bacterial contaminants was found. Spontaneous occurrence of foamy virus was recorded in some of the monkey-kidney cultures used for tests on the final vaccine of both 3C and 4A; it occurred in both control and test cultures, and gave some difficulty of interpretation. The comparable tests on the secondary seed lots passed satisfactorily.

Stability of Vaccines.—Ampoules of vaccines 3C and 4A were returned for titration from Nigeria after each vaccination session. No appreciable loss in virus titre was found. Ampoules of vaccine were exposed to sunlight in Nigeria at a temperature of 91° F. (32.8° C.) for 30 minutes and for 60 minutes. The loss in titre was 0.3 logs and 0.9 logs respectively.

Human Volunteers.—Before vaccines 3C and 4A were used in children, three adult volunteers were injected subcutaneously with each batch. No untoward reactions occurred.

Discussion

No specifications have yet been formulated for the preparation of measles vaccines. With few differences the procedures used for the vaccines described here are those described by Enders *et al.* (1960) for the clinical trial batches used in the United States in 1959 and 1960. Two techniques found to increase the yield of measles virus from the tissue cultures were used in the preparation of the two British vaccines: lowering the temperature of incubation of the tissue cultures to 32° C. during the period of virus growth, and freezing and thawing of the cultures to liberate cell-associated virus. The freeze-dried preparation appears to be stable under normal field conditions. Even after exposure of vaccine ampoules to strong sunlight little loss of titre occurred. The amount of virus in the preparation, together with the degree of stability shown, is sufficient to ensure a potent vaccine even when transported without refrigeration or subjected to adverse storage temperature.

In the absence of specifications for the testing of measles vaccine it was decided to test equally seed lots and final vaccine batches, following the principles established for yellow-fever vaccine and elaborated to include some tests necessary for poliovirus vaccine. The extensive testing of both seed and final vaccine is a necessity for attenuated live poliovirus vaccines because of the high risk of extraneous viruses in any individual monkey-kidney culture batch used for propagating the virus. Yellow-fever vaccine, on the other hand, is grown in chick embryos, a tissue with a very low incidence of viral contamination, and the burden of testing lies mainly on the seed lot. Since measles vaccine is also prepared from chick-embryo tissue, a similar principle may be held to apply in the future. However, for exploratory studies, such as those reported here, testing of both seed and final batch seems justified even to the extent of looking for known human pathogens never yet encountered in chick embryos. The inoculation of monkeys by the intracerebral route serves, for example, as a test for neurotropic viruses as well as an indicator of the virulence of the vaccine strain.

In respect of attenuation of the measles virus the monkey test has several difficulties. The animals may undergo natural measles infection prior to or at the same time as the actual carrying out of the test; the criteria of behaviour of virulent and attenuated measles strains are poorly defined; and the clinical symptoms not always easy to observe. While viraemia in inoculated monkeys is common with virulent strains and rare with attenuated, it is recorded in one monkey given attenuated virus by Enders *et al.* (1960), more frequently by Schwartz *et al.* (1960), and in one monkey in our series of tests. These findings might be regarded equally as indicating that viraemia is an unreliable index of attenuation or that the vaccine strain is insufficiently attenuated or that a variable incidence of wild infection was present in each group. In view of these difficulties it would appear a more reasonable and practicable objective to perform the monkey test only on seed lots on the model of yellow fever vaccine, at least when routine batch production is begun.

Summary

Descriptions are given of the origin and preparation of four batches of measles vaccine used in the clinical trials described in Parts II and III. The method of preparation and testing of the two batches prepared in Britain are given in detail. Some problems arising from individual tests are outlined and the principles underlying testing are discussed.

The vaccines consisted of infected chick-embryo-tissue-culture fluids. All four were of similar potency with from $10^{2.7}$ to $10^{3.4}$ TCD50 per human dose. Three vaccines were freeze-dried and the fourth was stored frozen at $-70°$ C. Freeze-dried vaccine was stable under field conditions and resisted exposure to sunlight.

We are indebted to Dr. V. Udall, of the Wellcome Research Laboratories, for histological examinations of inoculated animals; and to Dr. S. L. Katz for advice and strains of virus.

PART II. CLINICAL TRIAL IN NIGERIAN CHILDREN

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Clinical trials of measles vaccines prepared in the U.S.A. from the Edmonston strain of measles virus have been made in children in the United States (Katz *et al.*, 1960a, 1960b; Stokes *et al.*, 1960) and in Panama (Hoekenga *et al.*, 1960). Vaccination was followed by the formation of measles antibody and, in studies where exposure was demonstrated, by protection against the natural disease. The principal vaccination reactions consisted of pyrexia, which occurred in about four-fifths of the cases, and rash, which occurred in about half; children exhibiting these reactions were noticeably less ill than those with natural measles, and respiratory complications were minimal.

In the trial described here the clinical and antibody response to measles vaccine has been studied in children in Nigeria, among whom the mortality from measles is very high; the social and epidemiological circumstances in Nigeria differ greatly from those in the U.S.A. and Panama.

Procedure

There were three studies, each of which included a control group of children vaccinated with Salk type poliomyelitis vaccine. Three measles vaccines were used.