necessary to lay down the sequences of movement in advance.

It is not known whether this neurocellular atrophy is a direct result of the ageing process or whether it is based on ischaemia. Biemond (1951) found disappearance of Purkinje cells as a minimal result in some cases of basilar artery thrombosis, and suggested that "slight interference with the circulation in the cerebellum may lead to a local and perhaps also a more diffuse disappearance of the Purkinje cells." These observations are particularly relevant in view of the fact that no less than 50% of the whole sample and 60% of those having drop-attacks were unable to throw their head back without immediate symptoms. Reasons have already been adduced for the view that this is based on temporary obstruction to one or both vertebral arteries facilitated by senile cervical osteoarthritis, and it fits in well with Kremer's (1958) suggestion that drop-attacks are based on transient changes in the blood-supply to the brain-stem. Relative ischaemia in the territory supplied by the vertebral and basilar arteries might also underlie the high incidence of abnormal plantar responses, and much of the vertigo of which old people complain so bitterly may have the same origin.

It is suggested, therefore, that the general insecurity of postural control and the liability to fall which are characteristic of old age are based ultimately on a decline in the number of nerve cells in the brain-stem, cerebellum, and other centres—below that available for the maintenance of normal postural function in earlier life. The adverse effects of this cellular poverty will inevitably be accentuated by interference with the blood supply to the region which, in two ways, is particularly apt to happen in old age—by the liability of the vertebral arteries to temporary obstruction and the general proneness to phases of hypotension based on postural and other causes. Many intriguing problems remain; the drop-attack remains a remarkable event—from its instantaneous onset to its apparent path of recovery in some cases by the initiation, through pressure on the soles of the feet, of a reflex which seems hitherto to have belonged more to the province of neurophysiology than of clinical medicine. Therein, however, lies one of the fascinations of old age as a clinical study, for these phenomena have been uncovered because at this stage of life Nature resembles the engineer who may release the unexpected when he tests his materials to destruction.

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SEROLOGICAL RESPONSES AND CLINICAL REACTIONS TO INFLUENZA VIRUS VACCINES*

BY

F. HIMMELWEIT, M.D., Ph.D., F.R.C.P.Ed.
Director, Department of Virus Research, Wright-Fleming Institute of Microbiology, St. Mary's Hospital Medical School, London

In 1956-8 the Medical Research Council Committee on Influenza and Other Respiratory Virus Vaccines carried out a serological trial with oil-adjuvant vaccines of various viscosities, and in 1958–9 a trial with vaccines admixed with different amounts of aluminium phosphate.

In planning the 1956–8 trial, consideration was given to the fact that in past trials the very efficacious oil-adjuvant vaccines produced severe local reaction in a small number of individuals (Philip *et al.*, 1954; M.R.C. 1955, 1957). It was thought that, apart from purely chemical factors, the degree of viscosity of the vaccines, or possible physico-chemical changes arising during storage, might play a part in the aetiology of these reactions. However, before attempting to discover the influence of these factors on reaction rates in a large-scale trial, it was decided to study, with small groups of volunteers, the serological responses to fresh emulsified vaccines of different viscosities and to the same vaccines after storage.

In detail, the object of the trial was then: (1) To compare the serological responses, and as far as possible to observe clinical reactions, to three oil-adjuvant influenza virus vaccines which differed only in viscosity, and to compare these responses with the response to a saline vaccine containing the same quantity of antigen. (2) To assess serologically and by clinical observation the keeping properties of these three oil-adjuvant vaccines after storage for four weeks at 4° C.

The purpose of the 1958–9 trial was to examine, in view of a slight risk of provoking poliomyelitis especially in children, the value of aluminium phosphate in saline influenza virus vaccines. In all the earlier trials of saline vaccines which have been organized by the Committee aluminium phosphate was a component of the vaccines. It was thought that aluminium phosphate had possibly an adjuvant effect in man and that this substance was, at least in part, responsible for the low rate of both the local and the general reactions observed with aluminium-phosphate-adsorbed vaccines.

To ascertain the validity of these suppositions, it was decided: (1) To study in a serological trial, with small groups of volunteers, the comparative adjuvant effect, if any, of 10 mg., 5 mg., and 2.5 mg. of aluminium phosphate per dose of influenza virus vaccine, using as a control a vaccine without aluminium phosphate but containing the same quantity of antigen. (2) To compare the clinical reactions to these four vaccines in somewhat larger groups of volunteers.

Vaccines

The vaccines used in both trials were prepared at the Wright-Fleming Institute by methods described

*A report to the Medical Research Council Committee on Influenza and Other Respiratory Virus Vaccines.

previously (M.R.C., 1957) with, however, some modifications. One new technical feature was the use of an emulsifying machine specially developed for the preparation of oil-adjuvant vaccines.† The need for an apparatus that would bring about the complete emulsification of the vaccine components under sterile conditions and permit the production of emulsions, of predetermined viscosities, in the small quantities suitable for experimentation, arose from the fact that neither syringes, which were used hitherto, nor commercially available homogenizers met these requirements fully. Essentially, the emulsifier (Fig. 1), which is based on a well-known principle, is an electrically driven geared

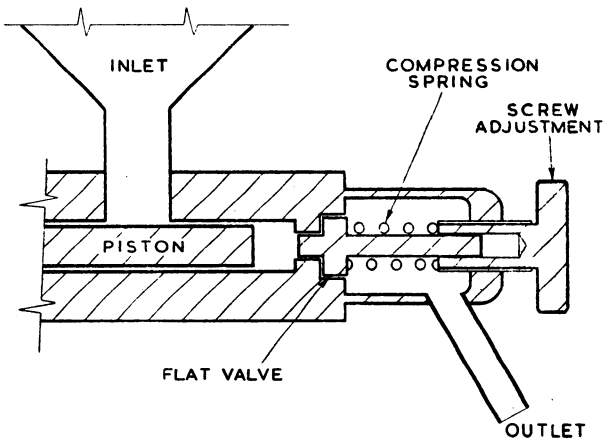

FIG. 1.—Diagram of the head of the emulsifying apparatus.

pump made of stainless steel. A reciprocating piston in the horizontal chamber of the pump forces the premixed component fluids of the vaccine through an aperture formed when the flat-headed outlet valve is lifted from its seating. The valve itself is controlled by a compression spring, the tension of which can be adjusted by a graduated control knob. The degree of tension of the spring determines the effective size of the aperture, and hence the viscosity of the emulsion.

The premixing of the fluids takes place in a conical glass funnel—which is provided with a stopcock and connected vertically to the chamber of the pump—by means of an electrically driven perforated stainless-steel stirrer. With the stopcock closed, a measured volume of a mixture of nine parts of a light mineral oil and one part of "arlacel A" (the emulsifying agent) is poured into the funnel. Then, with the stirrer set in motion, an equal volume of a virus suspension is added slowly to the fluid in the funnel. After thorough mixing, the stopcock is opened and the evenly turbid mixture flows into the horizontal chamber of the emulsifier. When the piston moves forward it closes the outlet of the funnel into the chamber, and then forces the mixture, now trapped in the chamber, through the outlet valve. On the return stroke of the piston the chamber refills when the piston has moved back far enough to allow the mixture to flow in again from the funnel.

The following vaccines were prepared for the serological trial of 1956–8:

Vaccine S.—A saline vaccine prepared from the A/England/211/56 strain of influenza virus A. It contained 2,000 haemagglutinating units of virus and 10 mg. of aluminium phosphate per dose (1 ml.).

†The apparatus was made by, and is available from, T. Giusti and Son Limited, 210/212 York Way, London N.7.

Vaccine Eh.—A water-in-oil emulsion of high viscosity, containing 2,000 haemagglutinating units of A/England/211/56 virus per dose (0.25 ml.).

Vaccine Em.—A water-in-oil emulsion of medium viscosity, containing 2,000 haemagglutinating units of A/England/211/56 virus per dose (0.25 ml.).

Vaccine El.—A water-in-oil emulsion of low viscosity, containing 2,000 haemagglutinating units of A/England/211/56 virus per dose (0.25 ml.).

Vaccine Eh'.—The same as vaccine Eh, but stored for one month at 4° C.

Vaccine Em'.—The same as vaccine Em, but stored for one month at 4° C.

Vaccine El'.—The same as vaccine El, but stored for one month at 4° C.

Because of favourable reports received from the U.S.A., the mineral oil "drakaol No. 6" was used instead of "bayol F" in the preparation of the emulsified vaccines. Supplies of this oil and of purified arlacel A were obtained from the U.S.A. by the Medical Research Council. The "high," "medium," and "low" viscosities of the emulsions resembled the viscosity of treacle, heavy mineral oil, and fairly light mineral oil respectively. After centrifuging for 10 minutes at 2,000 r.p.m., none of the emulsified vaccines showed any continuous aqueous phase, but some free oil was present in all of them, corresponding in amount to the viscosity of the emulsions—the lower the viscosity the greater the amount of free oil. There were no signs of breakdown in the stored emulsified vaccines.

For the serological trial of 1958–9, the following vaccines were made:

Vaccine A.—A saline vaccine prepared from the Asian strain A/Singapore/1/57. It contained 20,000 haem-agglutinating units of virus and 10 mg. of aluminium phosphate per dose (1 ml.).

Vaccine B.—A saline vaccine prepared from the Asian strain A/Singapore/1/57. It contained 20,000 haem-agglutinating units of virus and 5 mg. of aluminium phosphate per dose (1 ml.).

Vaccine C.—A saline vaccine prepared from the Asian strain A/Singapore/1/57. It contained 20,000 haem-agglutinating units of virus and 2.5 mg. of aluminium phosphate per dose (1 ml.).

Vaccine D.—A saline (control) vaccine prepared from the Asian strain A/Singapore/1/57. It contained 20,000 haemagglutinating units of virus (but no aluminium phosphate) per dose (1 ml.).

The virus suspension used in the preparation of these vaccines, like that in the 1956–8 trial, was highly purified by the differential centrifugation of extracts of the deposits recovered after centrifuging infective allantoic fluids. The final preparation was the supernatant suspension obtained after centrifuging the penultimate preparation for 10 minutes at 8,000 r.p.m. in a Spinco centrifuge.

Serological Procedures

All the pre- and post-inoculation sera of both trials were tested, at the Wright–Fleming Institute, for the presence of haemagglutination-inhibiting antibodies. To obtain independent confirmation, a small number of sera, chosen at random, were also examined at the World Influenza Centre, Mill Hill, or in the Virus Research Laboratory of the University of Sheffield. The results of these investigations were in agreement with those obtained at the Wright–Fleming Institute. The A/England/211/56 virus antigen for the inhibition tests of the sera from the 1956–8 trial was prepared

from the same seed virus used in the manufacture of the vaccines for the trial. This strain was not found to be affected by non-specific inhibitors. The Asian strain A/Singapore/1/57 which was incorporated in the vaccines for the 1958–9 trial proved, however, to be highly sensitive to non-specific inhibitors.

To obviate the necessity of treating the many sera of this trial with *Vibrio cholerae* filtrate to destroy these inhibitors, an Asian strain devoid of such sensitivity was obtained from the Central Public Health Laboratory, Colindale. This strain, which had been isolated from a Pakistani seaman in 1957, was not inhibited by human sera obtained before 1957. It indicated Asian antibodies, however, to almost the same titre as the A/Singapore/1/57 strain, in sera obtained in the 1958–9 trial that had been treated with *Vibrio cholerae* filtrate. When, instead of fowl cells, 0.5% human O cells were used in the test, the indicating properties of the Pakistani strain equalled those of the A/Singapore/1/57 strain. The use of human cells, because of their smaller size, less streamlined shape, and possible lower density, prolongs the usual reading-time of titrations from one to one-and-a-half hours. However, by using as a diluent 0.62% lithium chloride in M/100 phosphate buffer solution of *p*H 7 – 7.2, instead of the usual 0.85% sodium chloride solution buffered in the same way, it was found possible to overcome this drawback and to obtain, with human cells, patterns which permitted the determination of well-defined end-points after one hour.

Because of the lower density of the 0.62% lithium chloride solution, which is, by calculation, osmotically closely equivalent to 0.85% sodium chloride solution, various types of red cells were found in sediment in approximately two-thirds the time taken in the physiological saline. No untoward effects on haemagglutination by influenza viruses or on inhibition of haemagglutination by antibodies were observed with the lithium chloride solution. Occasionally, slipping of cells occurred in inhibition tests with this solution as it does with physiological saline. This was obviated, in repeat tests, by adding 0.1% bovine albumin (Armour) to the diluent. The physiological and physical properties of the lithium chloride solution suggest that its wider use in experimental and clinical pathology might prove advantageous.

In addition to the inhibition tests, complement-fixation tests were carried out at the Wright–Fleming Institute on all sera from the 1958–9 trial. A stock of particle antigen for the tests was prepared from a pool of allantoic fluid, infected with A/Singapore/1/57 strain, by differential centrifugation.

1956–8 Trial

Organization.—This trial was carried out in two parts. For both parts volunteers were recruited from the medical and nursing staffs of 11 mental hospitals in the Metropolitan and Epsom areas. In part I, volunteers, selected at random, were inoculated with the saline vaccine S or one of the emulsified vaccines Eh, Em, and El, in December, 1956. In part II, the emulsified vaccines, Eh′, Em′, or El′, were given four weeks later to other volunteers, similarly selected. Vaccine S was given by deep subcutaneous inoculation into the left upper arm; all other vaccines were injected deeply into the left triceps. In all, over 300 volunteers received vaccines, but only from 253 of them was it found possible to obtain complete sets of blood samples. These were taken before inoculation and then after six weeks, three months, six months, and one year. All volunteers were examined for reactions 48 hours after inoculation and on the days when the post-inoculation blood samples were taken.

Serological Results.—The geometric-mean antibody responses to the vaccines in parts I and II of the trial are depicted graphically in Figs. 2 and 3. It will be

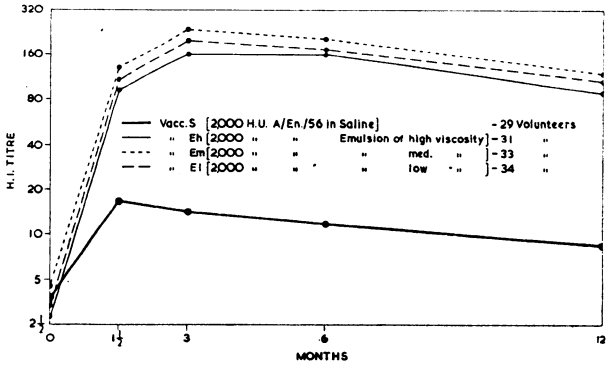

FIG. 2.—Comparison of geometric means of haemagglutination-inhibiting antibodies before and after administration of oil-adjuvant vaccines of various viscosities and of a saline vaccine.

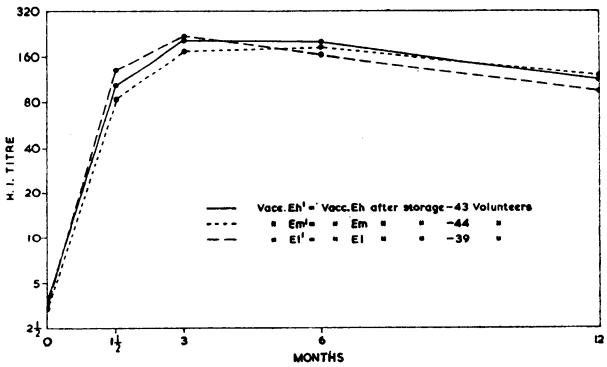

FIG. 3.—Comparison of geometric means of haemagglutination-inhibiting antibodies before and after administration of the oil-adjuvant vaccines referred to in Fig. 2, after storage for one month at 4° C.

noted that all emulsified vaccines evoked higher and more sustained antibody levels than the saline vaccine. This finding confirms previous observations (Salk *et al.*, 1952; M.R.C., 1955, 1957) on the adjuvant effect of a mineral oil in conjuction with arlacel A on the antigenicity of influenza virus antigens. It will also be seen from the graphs that the emulsified vaccines, when compared with each other individually or in the sets of three, produced very similar antibody responses. From these results the conclusions may be drawn (1) that the different degrees of viscosity of the three oil-adjuvant vaccines had no influence on their antigenicity, and (2) that these vaccines, when stored for one month at 4° C., did not lose potency.

Reactions.—Neither immediate nor delayed local reactions were observed in the volunteers who received the emulsified vaccines. The saline vaccine produced in a few cases mild, transitory, local reactions. There were no general reactions with any of the vaccines.

1958–9 Trial

Organization.—This trial was conducted with the co-operation of Surgeon Captain S. H. R. Price, R.N.,

principal medical officer of H.M.S. *Collingwood*, who, in October, 1958, arranged for 400 volunteers to be inoculated at random with vaccines A, B, C, and D. 85% of the volunteers were 18-25 years old; the remainder were over 35. All vaccines were given by deep subcutaneous inoculation. From approximately 40 members of each group of 100 volunteers who received one of the vaccines, blood samples were taken immediately before inoculation, and three weeks and three months after inoculation. A record sheet was kept for each volunteer, in which details of local and general reactions, if any, were entered. Each volunteer was examined for reactions after 24 and 48 hours, and further examinations were carried out at the end of the third week and third month after inoculation. To obtain uniformity of assessment the naval authorities were asked to arrange that the volunteers be inspected by the same medical officer throughout.

Serological Results.—It can be seen from Fig. 4, which shows the geometric means of the haemagglutination-inhibiting antibody titres of the four groups of volunteers before and after inoculation with the four vaccines, that aluminium phosphate had no adjuvant effect on the three vaccines which contained various amounts of that substance. This finding was confirmed by the results of the complement-fixation tests (Fig. 5).

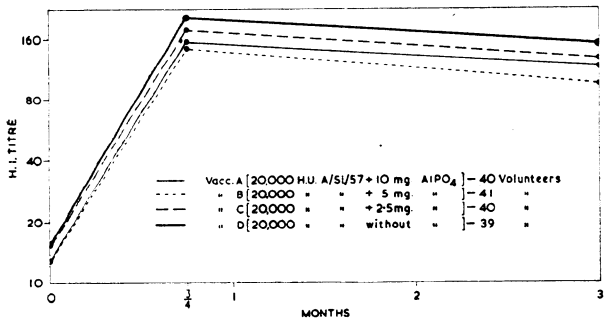

FIG. 4.—Comparison of geometric means of haemagglutination-inhibiting antibodies before and after administration of vaccines containing different amounts of $AlPO_4$ and of a control vaccine.

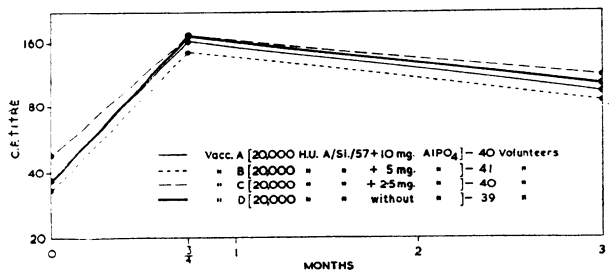

FIG. 5.—Comparison of geometric means of complement-fixing antibodies before and after administration of the vaccines referred to in Fig. 4.

Thus both tests showed that there was no difference between the antibody-evoking properties of the four vaccines.

Reactions.—The Table shows that only a few local and general reactions occurred in the four volunteer groups and that the slight differences between the groups are of little significance. It is clear that, at least under the conditions of this particular trial, aluminium phosphate could not be shown to reduce the small reaction rates.

Summary of Reactions to Three Influenza Virus Vaccines Containing Different Amounts of Aluminium Phosphate and to a Control Vaccine

Volunteers		Vaccine	$AlPO_4$ mg. Dose	Local Reactions				General Reactions			
No.	Age			Slight	Moderate	Severe	Total	Slight	Moderate	Severe	Total
87	18–25	A	10	1	—	—	1	—	—	1	1
86	18–25	B	5	5	—	1	6	2	2	—	4
85	18–25	C	2·5	1	—	—	1	—	—	—	—
85	18–25	D	0	3	—	—	3	—	—	—	—
13	35+	A	10	2	1	—	3	1	2	—	3
14	35+	B	5	—	—	—	—	—	—	—	—
15	35+	C	2·5	—	—	—	—	—	—	—	—
15	35+	D	0	—	—	—	—	—	—	—	—
100	All ages	A	10	3	1	—	4	1	2	1	4
100	All ages	B	5	5	1	—	6	2	2	—	4
100	All ages	C	2·5	1	—	—	1	—	—	—	—
100	All ages	D	0	3	—	—	3	—	—	—	—

Discussion

The results of both serological trials show that, while aluminium phosphate had no adjuvant effect on the antigenicity of influenza virus vaccines, the adjuvant mixture introduced by Freund *et al.* (1948a, 1948b) produced this effect to an impressive extent. The production of occasional severe local reactions, in the form of sterile abscesses, by this mixture is still the only contraindication to its wide-scale introduction as an adjuvant to vaccines in this country. The 1956–8 trial aimed at identifying a possible factor in the causation of such abscesses. It is conceivable that too viscous an emulsion may, by virtue of its enhanced foreign-body-like action, promote the formation of abscesses in especially sensitive individuals. The groups in the 1956–8 trial to whom emulsified vaccines of different viscosities were given were too small to provide an answer to this question. The serological results, however, permit the conclusion that, provided vaccines are well emulsified, those of low viscosity are as potent as highly viscous ones. It is hoped that in future trials this finding may lead to further tests.

The observation that well-emulsified vaccines show good keeping properties after storage for one month at 4° C. is of practical value because, if the necessity for large-scale production of emulsified vaccine should arise, the problem of lengthy storage before and after distribution of such vaccines should not prove insuperable.

That aluminium phosphate had no adjuvant effect in the volunteers, though it had this effect in mice (unpublished observation), is perhaps to be explained by the fact that it is more irritating to the tissues of the mouse than to those of man. After the subcutaneous injection of influenza vaccines that contained aluminium phosphate, many of the mice developed small sterile abscesses at the site of the injection. However, the mice received, per unit of body-weight, much larger amounts of aluminium phosphate, and this circumstance has therefore also to be taken into account when assessing the relative merits of aluminium phosphate as an adjuvant to influenza vaccines. Further, one must bear in mind that concentrated preparations of only one particular virus antigen were under investigation in the 1958–9 trial, and that the conditions for demonstrating an adjuvant effect of aluminium phosphate in man might perhaps be more favourable with vaccines pre-

pared from other strains, or with vaccines containing smaller amounts of antigen.

Equally interesting was the finding that aluminium phosphate did not reduce the reaction rate in the groups of volunteers who received vaccines containing that substance. The point has already been made that the reaction rates in all groups were very low. It is probable that aluminum-phosphate-adsorbed vaccines show low reaction rates not because of the presence of aluminium phosphate in the vaccines but because of the purity brought about by the use of this substance in their manufacture. Aluminium phosphate was introduced in the preparation of vaccines during the last war, when high-speed centrifuges were not available to me, on observing that it absorbs, rapidly and almost completely and specifically, many influenza A viruses from infective allantoic fluids at *p*H 6.5 (*cf.* M.R.C., 1953; Miller and Schlesinger, 1955; Burke *et al.*, 1957; Frommhagen and Knight, 1959). The resulting stable complexes are easily handled during the ensuing purification by low-speed centrifugation.

Because of the removal of most of the extraneous protein, aluminum-phosphate-adsorbed vaccines are relatively very pure. Thus it came about that the low reaction rates after the administration of a number of such vaccines were attributed to the presence of aluminium phosphate. It is now evident from the results of the 1958–9 trial that at least an A/Singapore/1/57 virus antigen, if highly purified by differential centrifugation, does not require the admixture of aluminium phosphate to exhibit benign properties. Such properties may, however, indicate a low toxicity of this particular strain, and it is therefore possible that antigens prepared from more toxic strains—even if very pure—might prove to be far less innocuous unless aluminium phosphate were present in the vaccines. Nevertheless, in view of a slight risk of provoking poliomyelitis by the use of a prophylactic that contains aluminium phosphate (M.R.C., 1956), it seems advisable, for the time being, not to incorporate aluminium phosphate in influenza virus vaccines that have been adequately purified by other means.

Summary

The Medical Research Council Committee on Influenza and Other Respiratory Virus Vaccines carried out a trial in 1956–8, with 253 volunteers, to compare the serological responses and clinical reactions to three oil-adjuvant influenza virus vaccines of various viscosities, both before and after storage, and to compare these responses with those to a saline influenza virus vaccine.

The results showed (1) that the emulsified vaccines (confirming earlier observations) evoked greater and more prolonged antibody responses than the saline vaccine containing the same quantity of antigen; (2) that the viscosity of the emulsified vaccines had no influence on their antigenicity; (3) that storage of the emulsified vaccines for one month at 4° C. did not adversely affect their potency; and (4) that the emulsified vaccines caused neither local nor general reactions, in contrast to the saline vaccine, which in a few cases produced mild local reactions.

In a trial in 1958–9, three saline influenza virus vaccines, containing different amounts of aluminium phosphate, and a control influenza virus vaccine were given each to 100 volunteers to determine whether aluminium phosphate reduced the reaction rate. The pre- and post-inoculation sera of approximately 40 volunteers from each group were tested for antibodies to study a possible adjuvant effect of aluminium phosphate.

It was found (1) that the presence of aluminium phosphate in the vaccines had no influence on the small reaction rates, and (2) that aluminium phosphate exerted no adjuvant effect on their antigenicity.

The conclusions arrived at in the discussion of these results were (1) that there might be an advantage in using emulsified influenza virus vaccines of low viscosity, and (2) that, provisionally, aluminium phosphate should not be added to already purified influenza virus vaccines.

A great debt of gratitude is owed to the former secretary of the committee, Brigadier A. E. Richmond, and to the many who have co-operated in organizing the two trials. Acknowledgments for help in obtaining volunteers, making arrangements for inoculations, or collecting blood samples are due, in particular, to the medical superintendents of 11 mental hospitals of the South-West Metropolitan Region; to Dr. I. H. Maclean, consultant bacteriologist to the Mental Hospitals' Group Laboratory, West Park Hospital, Epsom; and to Surgeon Captain S. H. R. Price.

The co-operation of the many volunteers has to be recorded with much gratitude; their participation was a *sine qua non* for the trials.

Especially appreciated, too, was the help given by Dr. A. Isaacs, of the World Influenza Centre, Mill Hill, and by Dr. D. Hobson, of the Virus Research Laboratory, University of Sheffield, who confirmed serological findings.

Finally, grateful acknowledgments are made to the staff of the Department of Virus Research, Wright-Fleming Institute of Microbiology, for their help in preparing the vaccines and in coping with the serological tests.

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"I have often told my students that the first chapter in our history books requires amendment in view of modern medical science. Stated scientifically the reason why Dr. Jan van Riebeeck founded a settlement at the Cape in 1652 was that vitamin C has such poor keeping qualities. He could not have put it quite like that, of course. But he and his employer, the Dutch East India Company, knew that scurvy, which caused such a high morbidity and mortality among the sailors during their long voyage around the Cape on the way to the East, could be cured and even prevented by a diet of fresh vegetables and fruit. They knew that the food they loaded up before they left Holland gradually lost some quality which was necessary to prevent the onset of scurvy. By the time they reached the Cape all the crew were suffering in varying degree from this disease. To proceed further without a supply of fresh food meant the death of many." (Professor E. H. Cluver, South African Institute of Public Health's *Special Sixtieth Anniversary Brochure, 1900–1960.*)