advised on statistical evaluation. One of us (A.D.T.) was in receipt of a University of Edinburgh Faculty of Medicine Graduate Research Scholarship.

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Simultaneous Administration of Live Measles Virus Vaccine and Smallpox Vaccine

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Brit. med. J., 1967, 2, 672-676

Smallpox has been virtually eradicated from Europe and America by means of efficient control measures practised over many years. The disease is, however, still prevalent in large areas of Africa and Asia, and the possibility of global eradication was discussed by an expert committee of the World Health Organization (W.H.O., 1964).

Measles is also a serious disease in developing countries (Gans *et al.*, 1961; Morley *et al.*, 1963; Hendrickse and Sherman, 1965), having many clinical similarities to the illness as seen in Europe in the early part of the present century (Morley, 1964).

During the past few years methods of active immunization against measles have been developed (W.H.O., 1963). Much emphasis has been placed on the use of live vaccines, and there have been numerous reports on the use of the attenuated types of measles vaccines (Collard *et al.*, 1961; Hendrickse *et al.*, 1964; Krugman, 1965; Smorodintsev *et al.*, 1965; Rey *et al.*, 1965b), as well as on the comparison between *attenuated* and *further attenuated* vaccine strains with regard to clinical reactions and serological response (Du Pan, 1965; Hendrickse *et al.*, 1964, 1965, 1966a, 1966b).

Satisfactory results have been reported from Senegal and Nigeria in trials of further attenuated measles vaccines administered by needleless injectors (Rey *et al.*, 1965a; Hendrickse *et al.*, 1966a). It is clear that this method of injection would be required in any large-scale vaccination programme.

Plans for the eradication of smallpox and the control of measles in the near future, in 19 West African countries, "sponsored by the U.S. Government in co-operation with the World Health Organization," announced recently in the Nigerian press (*New Nigeria*, 1966; *Daily Times*, 1966), bring the problem of the simultaneous use of measles and smallpox vaccines to the forefront.

Meyer *et al.* (1964) reported on the response of 545 children in the Upper Volta who were given combined measles, smallpox, and yellow fever vaccines, which were physically mixed before being used in a single injection. They gave little detailed

information about the clinical responses to the procedure apart from that concerning febrile responses and rashes. Budd *et al.* (1965) evaluated measles and smallpox vaccines administered simultaneously to 258 children in Togoland; information, however, is not available at present whether or not the vaccines were physically mixed. The attenuated Enders B strain of measles vaccine was used in both trials.

The results of the trials indicated that antibody responses were good, but were accompanied by a high rate of severe febrile reactions. Similar trials have not been carried out in other West African territories, nor has there been a direct comparison between the responses to attenuated and further attenuated measles vaccine when given simultaneously with smallpox vaccine.

If developing countries are to embark on mass smallpox/measles vaccination campaigns information is urgently needed regarding the nature of reactions that are likely to follow the procedure. We need also to know which of the live measles vaccines currently available are most suitable for use in such a campaign in developing African countries.

In an attempt to answer these questions a clinical trial in children was undertaken in a town in Western Nigeria. In this trial one group was given *attenuated* measles vaccine plus smallpox vaccine; a second group was given *further attenuated* measles vaccine plus smallpox vaccine; and a third group, which acted as controls, was given smallpox vaccine only. Our aim was to determine the pattern of reaction to the simultaneous vaccination procedure, including antibody responses, and to discover whether there were any significant differences between reactions provoked by the attenuated or further attenuated measles vaccines when given with smallpox vaccine. We report here the results of the trial.

Material and Methods

The study was undertaken at Ipetu Ijesha, a town of some 43,000 to 47,000 persons, situated about 97 miles (156 km.) from Ibadan, in the Western Provinces of Nigeria.

Healthy children between the ages of 6 months and 3 years who had had neither measles nor smallpox vaccination were

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Temperature Responses

Table II and Figs. 1, 2, and 3 give details of temperature responses in the three groups. The following points are worthy of note:

1. Daily mean temperatures were significantly higher in the Rubeovax group than in the Wellcovax group and the control group (Fig. 1).
2. When the daily mean temperatures are plotted showing two standard deviations of the mean for the Rubeovax and Wellcovax groups, there is a statistically significant difference between the two groups (Fig. 2).

FIG. 2.—Mean daily temperatures, showing the standard deviations of the means (hatched areas indicate two standard deviations from means).

3. The incidence of pyrexia of 101° F. (38.3° C.) or over was significantly higher in the Rubeovax group than in the Wellcovax group ($P < 0.05$). The difference between these two groups is even more pronounced when considering temperatures of 103° F. (39.4° C.) or more ($P < 0.01$) (Table II; Fig. 3).

FIG. 3.—Percentage incidence of fever of 103° F. (39.4° C.) and over.

4. The difference between pyrexias recorded in the Wellcovax group and the smallpox only group were not statistically significant ($P > 0.05$).

5. There were 44 child/day attendances with fever of 103° F. (39.4° C.) and over out of 614 child/day attendances among the Rubeovax group. Corresponding figures for the Wellcovax group were 13 out of 680 child/day attendances, and 6 out of 304 child/day attendances for the smallpox only group. The difference

between the Rubeovax group and the other two groups is highly significant ($P < 0.001$).

Treatment for children with fever of 103° F. (39.4° C.) and over was tepid sponging and aspirin. In the Rubeovax group 16 children required tepid sponging—one of them on four consecutive days, two on three consecutive days, two on two consecutive days, and the remainder on one occasion only. In the Wellcovax group eight children required tepid sponging, two of them on two consecutive days; four children in the smallpox vaccine group required this treatment on one day only. The difference in the treatment rate between the Rubeovax group and the other two groups is significant at the 95% level of confidence.

Clinical Findings

Table III compares the incidence of various clinical reactions among the three groups. The significant finding in this Table is the higher proportion of children assessed as "unwell" in the Rubeovax group as compared with the other two groups ($P < 0.05$).

TABLE III.—Clinical Reactions

	Rubeovax Group (93)	Wellcovax Group (99)	Smallpox Only Group (45)
% with rash	44.1	40.4	15.5
" " and fever of 100° F. and over	43.0	39.4	13.3
% with cough	34.4	38.4	35.5
" " coryza	73.1	61.6	62.2
" " conjunctivitis	2.1	4.0	4.4
" " diarrhoea	29.0	19.2	20.0
" " faucial inflammation*	31.2	16.2	0
" " natural measles	1.1	0	2.2
" " malaria	1.1	3.0	0
" " smallpox vaccination take	97.8	96.9	100
" assessed as unwell†	25.8	11.1	8.9

* S.E.D. between the Rubeovax group and the Wellcovax group 6.11 ($P < 0.05$).

† S.E.D. between the Rubeovax group and the Wellcovax group 5.53 ($P < 0.05$). The reasons for an assessment of unwell are given in separate tables.

It will be noted that some of the conditions for which children were assessed as unwell were clearly not related to the vaccination procedure. Three of the 24 "unwells" in the Rubeovax group, 5 of the 11 in the Wellcovax group, and all four in the smallpox only group fell into this category (Table IV).

TABLE IV.—Reasons for Assessing Children as "Unwell"

	No. of Children		
	Rubeovax Group	Wellcovax Group	Smallpox Only Group
U.R.T.I.	6 (6)	1	
" and diarrhoea ± fever	3 (2)		
" diarrhoea, and vomiting	1 (1)		
" and doubtful convulsion	1 (1)		
" suspected L.R.T.I.	1 (1)		
L.R.T.I.			1 (1)
Convulsion, spasmophilia, and L.R.T.I.		1 (1)	
Very irritable; doubtful convulsion		1	
Dysentery		1 (1)	
Diarrhoea and generally unwell	2 (2)	1 (1)	
" dactylitis (known S.C.A.)	1 (1)		
Malaria and diarrhoea	1 (1)		
" diarrhoea, and vomiting, possible		1 (1)	
otitis media		1 (1)	
Malaria			
Otitis media	3 (3)		
S.F.R. on 3 consecutive days	2		
General ill-health; no specific cause	1 (1)		
? Natural measles	1 (1)		
Natural measles			1 (1)
Boils, ulcers, and skin sepsis	1 (1)		
Old cancerum oris		1 (1)	
Conjunctivitis ? aetiology			1 (1)
Stomatitis			1 (1)

U.R.T.I. = Upper respiratory tract infections and faucial inflammation.

L.R.T.I. = Lower respiratory tract infections.

S.C.A. = Sickle-cell anaemia.

S.F.R. = Severe febrile response—that is, fever of 103° F. (39.4° C.) or over.

The diagnosis of malaria was made by examination of blood films for the presence of the parasite.

Figures in parentheses are the numbers of children given specific treatment—that is, long-acting penicillin, tetracycline, chloramphenicol, sulphadimidine, and chloroquine.

Faucial inflammation was observed in 31.2% of the Rubeovax group and in 16.2% of the Wellcovax group. No faucial inflammation was noted in the smallpox only group. The difference between the incidence of faucial inflammation in the Rubeovax and the Wellcovax groups was statistically significant ($P < 0.05$).

A number of parents complained of "twitching" or "jerking" in their children, usually associated with high fever. Five in the Rubeovax group and three in the Wellcovax group complained of this symptom, but none in the smallpox only group. Careful questioning of the mothers of these children usually failed to elicit evidence of a true convulsion. It was concluded that these symptoms were a manifestation of restlessness associated with fever, and not genuine convulsions.

One patient in the Wellcovax group developed convulsions and tetany on the second day after vaccination and was later admitted to the Wesley Guild Hospital. This patient had received native medicine. Spasmophilia persisted for several days. The patient responded slowly to treatment with calcium gluconate and sedatives. He was given antibiotics for a respiratory infection which developed in hospital, and recovered completely. The nature of the symptoms in this patient and their time of onset suggest that this illness was incidental and not related to vaccination.

Response to Smallpox Vaccination

The incidence of successful primary vaccinations was 97.8% in the Rubeovax group, 96.9% in the Wellcovax group, and 100% in the smallpox only group (Table III). From questioning the mothers it seems likely that at least two of the children who failed to "take" may in fact have missed their smallpox vaccine injection. There were only five children who failed to "take" and four of them were aged 2 or more. There was no evidence of significant interference between the measles and smallpox vaccines.

The great majority of children developed lesions of 1 cm. or less in diameter which showed the classical characteristics of primary vaccination.

Antibody Responses to Measles Vaccination

Antibody responses determined on paired serum samples by the haemagglutination inhibition method (Hendrickse *et al.*, 1966b) gave the following results:

Rubeovax Group.—There were 80 paired serum samples, of which 6 (7.5%) showed antibodies in the first sample. Of the 74 initially nonimmune children 93.2% showed satisfactory antibody conversions. The geometric mean titre of the post-vaccination samples was 140.

Wellcovax Group.—There were 91 paired serum samples, of which 10 (11.0%) showed antibodies in the first sample. Of the 81 initially nonimmune children 87.6% showed satisfactory development of measles antibody. The geometric mean titre of the postvaccination samples was 89.

Discussion

Findings in this trial clearly show that clinical reactions to simultaneous measles and smallpox vaccination are more frequent and severe with *attenuated* measles vaccines than with *further attenuated* measles vaccines. The differences observed in temperature responses and "unwell" rates in the two measles vaccine groups in this trial are statistically significant.

Serological findings, on the other hand, confirm that both measles vaccines used are effective immunizing agents. The

overall antibody conversion rates were similar in the two measles vaccine groups. The difference observed in the geometric mean titres of measles antibody in the postvaccination bloods between the attenuated and further attenuated measles vaccine groups accords with findings reported in other trials in which these vaccines were compared. It has not been demonstrated to date that the difference in geometric mean titres achieved by the two classes of live measles vaccine reflects a difference in the protection afforded against the disease.

The subjects in this trial were selected healthy children who were protected from malaria during the period of the trial. It can be anticipated that reaction rates in unselected groups of children not protected from malaria will probably be somewhat higher than those recorded here. We expect, therefore, that if mass measles plus smallpox vaccination is undertaken in Nigeria with attenuated measles vaccine significant reactions will be provoked in more than 30% of the children vaccinated.

Our findings in this respect are similar to those observed in a combined measles plus smallpox vaccination study undertaken recently in Togoland (Budd *et al.*, 1965). If, on the other hand, mass vaccination is undertaken with further attenuated measles vaccine the anticipated incidence of significant reactions will be in the region of approximately 10%. This reaction rate is very similar to that observed in our group of children who received only smallpox vaccine.

Medical services in developing countries could easily be disorganized by a sudden general increase in demands for medical attention by a section of the population. If mass measles plus smallpox vaccination were undertaken in such countries it must be anticipated that in the immediate postvaccination period there would be an increased demand for medical attention. The size of this increase would be directly proportional to the reactogenicity of the vaccines employed. In the light of our experience it can be estimated that the use of attenuated measles vaccines will create at least a threefold greater demand for medical attention than the further attenuated measles vaccines.

Our findings indicate that simultaneous measles and smallpox vaccinations on a large scale would be a justifiable procedure if further attenuated measles vaccine strains were used.

Summary

The need for a trial comparing the *attenuated* and *further attenuated* measles vaccines given simultaneously with smallpox vaccine is discussed.

A comparative trial of attenuated measles vaccine given simultaneously with smallpox vaccine, further attenuated measles vaccine with smallpox vaccine, and smallpox vaccine given alone is reported. Clinical assessment of 237 children showed that those who received attenuated measles vaccine plus smallpox vaccine had significantly higher fever and morbidity rates than those given either further attenuated measles vaccine plus smallpox vaccine or smallpox vaccine alone. The measles antibody responses in initially susceptible children were satisfactory in both groups given measles vaccine. The incidence of successful primary smallpox vaccinations was between 97 and 100% in all groups. The results of this trial show that a further attenuated measles vaccine given simultaneously with smallpox vaccine would be preferable to an attenuated measles vaccine given with smallpox vaccine in a developing country.

We wish to thank the Permanent Secretary, Ministry of Health, Western Provinces of Nigeria, for permission to carry out this trial; His Highness the Apetu of Ipetu for securing the co-operation of the local population and the help and hospitality of the staff of the Save the Children Fund organization at Ilesha, without which the trial could not have been undertaken. We are grateful to the medical superintendent and the laboratory, nursing, and auxiliary staff of the Wesley Guild Hospital, Ilesha, for their co-operation,

and the departments of University College Hospital, Ibadan, who gave their assistance.

The Wellcome Foundation kindly supplied the vaccines and the needleless injectors used in this trial.

Reprints of this paper will be available from Professor R. G. Hendrickse, Department of Paediatrics, U.C.H., Ibadan, Nigeria.

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Intestinal Hormones and Plasma Insulin: an Insulinotropic Action of Secretin*

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Brit. med. J., 1967, **2**, 676–678

McIntyre, Holdsworth, and Turner (1964) and Elrick, Stimmler, Hlad, and Arai (1964) demonstrated the greater effect of oral glucose administration, compared with intravenous, in stimulating a rise in plasma insulin levels. McIntyre, Holdsworth, and Turner (1965) presented evidence that the augmentation of the response was due to the release of a hormone from the gut wall. At present there are two obvious candidates for this role—secretin and glucagon (*Lancet*, 1966). We have already published evidence that there is no rise in fasting venous plasma immunoreactive insulin levels when endogenous secretin release is stimulated by intraduodenal instillation of acid (Boyns, Jarrett, and Keen, 1966). In this paper we report further studies of the effect of endogenous secretin on the plasma immunoreactive insulin response to intravenous glucose. In addition, the effects of purified porcine secretin have been studied and compared with those of purified cholecystokinin/pancreozymin.

Materials and Methods

Endogenous secretin was stimulated by intraduodenal infusion of 0.05 N citric acid (pH 2.5), 5 ml./minute for alternate minutes over 20 minutes. Purified secretin (20,000 clinical units/mg.) and purified cholecystokinin/pancreozymin—referred to subsequently as C.C.K.—(1,500 Ivy dog units/mg.) were obtained from Professor J. E. Jorpes and Dr. V. Mutt; 6 µg. of secretin or 50 µg. of C.C.K. was made up in 20 ml. of physiological saline and injected intravenously at a rate of 1 ml./minute.

* Based on a communication to the Medical and Scientific Section of the British Diabetic Association, 30 September 1966.

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Intravenous infusions of 5% glucose were administered at a rate of 20 g. of glucose per hour. Preliminary experiments had shown that this rate of infusion provoked only a small and slow rise in plasma immunoreactive insulin levels. When two infusions were given on the same day, two hours elapsed between the end of the first and the beginning of the second infusion.

Blood samples were taken into heparinized tubes from an indwelling polyethylene cannula placed in an antecubital vein. Then 0.1 ml. was transferred to 3 ml. of 1% potassium fluoride for subsequent estimation of blood sugar on an autoanalyser, the method of Hoffman (1937) being used. Plasma was separated by centrifugation and was assayed for insulin by a double antibody precipitation method similar to those described by Morgan and Lazarow (1963) and Hales and Randle (1963).

All the experiments reported were performed in the morning, after an overnight fast, on five healthy men aged 25–40, who lay at rest during the experimental period. All had normal oral glucose tolerance.

Results

In three subjects the blood sugar and plasma immunoreactive insulin response to intravenous glucose infusion was measured with an intraduodenal tube in position. In the control infusion, intraduodenal infusion of physiological saline, 5 ml. per minute for alternate minutes, was carried out for the first 20 minutes of the glucose infusion. The results were compared with a similar infusion of citric acid (Fig. 1). In none of the three subjects did the infusion of citric acid augment the response of the plasma immunoreactive insulin to intravenous glucose.

Secretin or C.C.K. was infused intravenously, alone (Fig. 2) and along with intravenous glucose (Fig. 3). In neither case