A Study of the Effect of Simultaneous Vaccination with BCG and Smallpox Vaccine in Newborn Infants*

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In order to determine whether the BCG and smallpox vaccinations of newborn infants could be combined, thus saving staff, time and money, particularly in the developing countries, 696 newborn infants in Taiwan were divided into three groups and vaccinated by one of the following three methods: simultaneous vaccination with BCG and smallpox vaccine, vaccination with BCG only, and vaccination with smallpox vaccine only. On follow-up, no difference was observed between the "simultaneous" group and the "smallpox only" group in respect of either no-take rate and frequency distribution by size of vaccination lesions or reactions to smallpox revaccination at 12 weeks. Tuberculin tests at 12 weeks revealed a normal distribution in the size of tuberculin reactions with a mean of 11.94 mm and 12.19 mm respectively in the "simultaneous" group and the "BCG only" group; in the "smallpox only" group all the reactions were below 9 mm, the great majority being 0-3 mm. The author concludes that there is no interaction between primary BCG and smallpox vaccination when both vaccines are administered simultaneously, and that, despite the reported complications, simultaneous administration of BCG and smallpox vaccine in newborn infants is a safe procedure.

As knowledge of disease processes has advanced, it has become apparent that a number of infectious diseases are now preventable through immunization during early infancy. Examples are smallpox, tuberculosis, pertussis, diphtheria, tetanus, and poliomyelitis. Thus, infants are now given several types of vaccination during a relatively short period of time after birth, and health workers in the community are deeply involved in these vaccination programmes. For this reason, there is a pressing need to integrate various types of vaccination, with a view to reducing the time it takes to administer them and also the number of attendances at health centres.

It has long been thought that some of these vaccinations may be combined, thereby saving staff, time and money. In the developing countries, where the resources, including manpower and funds for health services, are rather limited, such a procedure is most desirable. Integration of BCG vaccination with smallpox vaccination would be a step in this direction, as both are best given during the first

few months of life. If this integration were possible, BCG vaccination could take advantage of the popularity of smallpox vaccination, which has been established for many decades in many countries.

From an extensive review of the literature, Møller (1957) concluded that "there are conflicting opinions as regards the effect of simultaneous BCG and smallpox vaccination. In general and on theoretical grounds opinion is against this procedure, whereas the few people who have investigated the method in practice report satisfactory response and no complications". However, he admitted that "little factual knowledge is available on the effects of simultaneous or combined BCG and smallpox vaccination", and pointed out that "the experimental design of the studies conducted so far did not take into account essential statistical principles (there were, for example, no suitable control groups and no systematic exclusion of bias in reading and interpreting the results)".

Recently, Kawasaki (1959) and Huang (1962) conducted animal experiments and independently reached the conclusion that simultaneous vaccination with BCG and smallpox vaccine in guinea-pigs gives satisfactory immunological results similar to those obtained with independent BCG or smallpox

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vaccination, and that it does not lead to competition between antigens, or to an increase in complications.

Shimokawa (1960) made a study of a relatively small number of infants under the age of one-and-a-half years, using dried BCG and smallpox vaccine, and came to the conclusion that, no matter whether the vaccines were given in combination or separately at the same time, the results were no different from those obtained when single vaccination was given independently with the same types of vaccine.

Moodie & Cheng (1962) studied the effects of the concurrent BCG and smallpox vaccinations they had been giving since 1952 to more than 300 000 newborn babies in Hong Kong without recorded mishaps, and concluded that "the BCG has no effect on the local smallpox vaccination reaction or the 'take' rate, and on the other hand Mantoux conversion and BCG complications compare not unfavourably with results obtained in other parts of the world where economy is a less important consideration".

Because the above-mentioned studies had certain statistical disadvantages, the present investigation has been conducted along rather different lines, special attention being given to the evaluation of post-vaccination tuberculin allergy, to reactions to smallpox revaccination, and to a possible interaction between BCG vaccination and primary smallpox vaccination.

MATERIALS AND METHODS

Study population

The study population was newborn infants born in the following maternity wards or hospitals in Taipei City, Taiwan, during the period from 25 April 1962 to 28 July 1962:

- (1) the Provincial Taipei Maternity and Gynaecological Hospital,
- (2) the maternity ward of the National Taiwan University Hospital,
- (3) the maternity ward of the 801 Army Hospital.

Infants with a body-weight of less than 2500 g, those with apparent eczema, or those with a body temperature higher than 37.5°C were not given vaccination. Vaccination was given with the mother's consent, and not less than 24 hours after birth.

Experimental design

The study population was divided into three groups, which were given:

- (1) simultaneous vaccination with BCG and smallpox vaccine ("simultaneous" group);
- (2) smallpox vaccination only ("smallpox only" group);
- (3) BCG vaccination only ("BCG only" group).

The vaccination scheme was so arranged that the same type of vaccination was used in each of the hospitals for one week at a time (two visits a week per hospital for vaccination), so as to ensure the even spread of the three methods of vaccination over the three participating hospitals.

Technique of vaccination and vaccine products employed

BCG vaccination. 0.1 ml of 0.5 mg/ml liquid BCG vaccine prepared freshly (not older than two weeks) was given by intradermal injection in the deltoid region of the left arm. Six batches of BCG were used. The viable counts of these batches of vaccine were: 28.5, 23.3, 22.9, 26.1, 4.8, and 6.3×10^6 .

Smallpox vaccination. Liquid vaccine, Lot No. 292B, which was produced on 12 March 1962 and kept below 8°C in the refrigerator, was used throughout the study period. The same lot was also used for follow-up revaccination at 12 weeks. Vaccination was given by the scratch method at the deltoid insertion of the right arm. After two drops of vaccine had been dropped on to the skin, two linear scratches, 3 mm in length and 2 cm apart, were made with a sewing needle. Revaccination was given by the same technique, except that the site used for vaccination was the outer side of the left thigh.

Tuberculin testing. 5 TU of PPD RT 23 with Tween 80 were applied by intradermal injection. Readings were taken after 72 hours, and the size of reaction was measured and recorded.

Follow-up scheme

The follow-up study consisted of observations of smallpox vaccination lesions at seven days and of BCG vaccination lesions at four weeks and 12 weeks, tuberculin testing and smallpox revaccination (or vaccination) at 12 weeks with subsequent reading of tuberculin reactions after 72 hours, and observation of the reaction to smallpox revaccination (or vaccination) at three days and seven days. The axillary region of the vaccinated side was palpated at each visit for possible lymph-gland enlargement, and attention was also paid to possible complications.

TABLE 1
NUMBER OF INFANTS BORN AND NUMBER VACCINATED AT EACH OF THREE HOSPITALS,
BY TYPE OF VACCINATION

Hospital	Number born	Number vaccinated				Number not vaccinated
		Total	Simultaneous BCG and smallpox	Smallpox only	BCG only	
NTU Hospital	479	395	140	104	151	84
801 Army Hospital	483	432	157	154	121	51
Maternity Hospital	432	274	77	82	115	158
Total	1 394	1 101	374	340	387	293

The follow-up at seven days and four weeks took the form of home visits, while the follow-up at 12 weeks took the form of written requests by the clinic to the infants' parents for the children to be brought to the clinic. Home visits were paid to defaulters only.

Personnel

Whenever possible, all vaccinations were given immediately after birth and by the same nurse who also did the tuberculin testing and smallpox re-vaccination (or vaccination), read the tuberculin reactions, and observed the local reaction to smallpox revaccination at 12 weeks. The observations at seven days and four weeks were made by another nurse. One medical officer supervised the whole study and, on request by the nurses, examined the infants for possible complications.

At the time of reading of tuberculin reactions, the nurse did not know which method of vaccination had

been used, so that any bias in reading the reactions was excluded.

Analysis of material

During the three-month period from 25 April 1962 to 28 July 1962, 1394 infants were born at the three participating hospitals. Of these, 293 were not vaccinated either because the mother refused or else because the infants were under weight; the remaining 1101 were each given one of the three types of vaccination. The distribution of infants by hospital and type of vaccination is shown in Table 1.

Table 2 shows the number of infants vaccinated, by residence and eligibility for home visits. All infants living in Taipei City were supposed to be followed up while, of the infants residing outside the city, only those in the near-by townships were eligible for home visits. Thus, of the 1101 infants vaccinated, 317 were not eligible for home visits and, of the remaining 784, 88 could not be located, the com-

TABLE 2
NUMBER OF INFANTS VACCINATED, BY RESIDENCE AND ELIGIBILITY
FOR HOME VISIT

Residence	Number vaccinated	Number eligible for home visit			Number not eligible for home visit
		Total	Located	Not located	
Taipei City	631	629	554	75	2
Outside the city	470	155	142	13	315
Total	1 101	784	696	88	317

TABLE 3
NUMBER AND PERCENTAGE OF INFANTS VACCINATED, BY TIME LAPSE AFTER BIRTH
AND TYPE OF VACCINATION

Time of vaccination	Total		Simultaneous		Smallpox only		BCG only	
	No.	%	No.	%	No.	%	No.	%
Within 24 hours	9	1.3	5	2.0	2	1.0	2	0.8
24-48 hours	254	36.5	99	39.4	68	34.0	87	35.5
49-96 hours	329	47.4	111	44.3	96	48.0	122	49.8
After 96 hours	104	14.8	36	14.3	34	17.0	34	13.9
Total	696	100.0	251	100.0	200	100.0	245	100.0

pleteness of the follow-up being 88.8%. The remaining 696 infants were successfully visited at one week and formed the main subjects of this investigation.

The analysis of the time of vaccination after birth is shown in Table 3. Only nine infants were vaccinated less than 24 hours after birth; the great majority (83.9%) were vaccinated at 24-96 hours. There is no significant difference between the three vaccination groups in the distribution of infants by time lapse after birth.

Analysis of the mothers' age and parity revealed no significant difference between the three vaccination groups.

It was inevitable that a small proportion of infants were lost between the different phases of follow-up for one reason or another. The number of infants followed up at different intervals for the three vaccination groups is shown in Table 4—82 infants had been lost by the time of the 12-week follow-up. The reasons for this loss are analysed in Table 5.

TABLE 4
NUMBER OF INFANTS FOLLOWED UP AT 7 DAYS,
4 WEEKS AND 12 WEEKS, BY TYPE OF VACCINATION

Time of follow-up	Simultaneous	Smallpox only	BCG only
7 days	251	200	—
4 weeks	242	—	245
12 weeks	225	175	214

RESULTS

Smallpox vaccination

Table 6 shows the number and percentage of infants in whom vesiculation was present at one week, and, for those with vesiculation, also the number of lesions in the "simultaneous" group and the "smallpox only" group. It is seen that 61 out of 251 (24.3%) in the "simultaneous" group, and 43 out of 200 (21.5%) in the "smallpox only" group

TABLE 5
NUMBER OF INFANTS LOST AT 12-WEEK FOLLOW-UP,
BY REASON AND BY TYPE OF VACCINATION

Reason	Total	Simultaneous	Smallpox only	BCG only
Died	2	1	1	—
Moving of family	8	4	2	2
Received BCG or smallpox vaccination during the intervening period	16	3	7	6
Parent's refusal of smallpox revaccination	4	1	3	—
Illness not related to vaccination	6	3	1	2
Absent from Taipei	3	1	1	1
Not located	43	13	14	16
Total	82	26	29	27

TABLE 6
RESULTS OF SMALLPOX VACCINATION AT ONE WEEK

Group	Total number of infants		Vesiculation present				Vesiculation absent	
			One lesion		Two lesions			
	No.	%	No.	%	No.	%	No.	%
Simultaneous	251	100.0	78	31.1	112	44.6	61	24.3
Smallpox only	200	100.0	75	37.5	82	41.0	43	21.5

$$\chi^2 = 2.35, \quad 0.368 > P > 0.223.$$

showed no take; about one-third of both groups developed one lesion, and the rest two lesions.

Table 7 shows the distribution, by size, of positive smallpox vaccination lesions at one week. It is seen that the "simultaneous" group and the "smallpox only" group show an almost identical type of distribution with mean sizes of 7.38 mm and 7.09 mm respectively, the difference being statistically insignificant.

BCG vaccination

As seen in Table 8, both the "simultaneous" group and the "BCG only" group show the same distribution pattern in the size of BCG vaccination lesions, with mean sizes of 4.95 mm and 5.19 mm respectively, the difference not being statistically significant. Apart from those with no take at initial smallpox vaccination in the "simultaneous" group, those with previous take are also not significantly different from the "BCG only" group in the distribution by size of BCG lesions, and in their means. Most of the lesions in both groups are at the stage of induration, a few having a small pustule.

Table 9 gives the distribution of BCG lesions by size at 12 weeks for both groups. It is readily seen that the distribution is similar and that the respective means are not significantly different. As compared with the results at four weeks, the mean size of the BCG lesions has decreased by approximately 1.1 mm in both groups, and almost all lesions have reached the stage of scarring.

Tuberculin testing

The frequency distribution of the tuberculin reactions at 12 weeks is shown for all three vaccination groups in Table 10 and in the figure. There is a distinct difference between the distribution pattern of the two groups that included BCG vaccination

and that of the "smallpox only" group: whereas the "simultaneous" group and the "BCG only" group showed almost identical unimodal normal distribution, all the reactions in the "smallpox only" group were below 10 mm, the great majority being 0-1 mm or 2-3 mm. The mean sizes of the tuberculin reactions were 11.94 mm and 12.19 mm for the "simultaneous" group and the "BCG only" group respectively.

Excluding those with no previous take in the "simultaneous" group, those with take at initial smallpox vaccination were also not significantly different from the "BCG only" group in the distribu-

DISTRIBUTION OF TUBERCULIN REACTIONS BY SIZE AT 12 WEEKS

TABLE 7
DISTRIBUTION OF POSITIVE SMALLPOX VACCINATION LESIONS,
BY SIZE AT ONE WEEK

Size of lesion (diameter in mm)	Total number of lesions		One lesion	Two lesions	
	No.	%		Lesion I	Lesion II
A. Simultaneous vaccination					
0-1	0	0	0	0	0
2-3	3	1.0	0	1	2
4-5	41	13.6	11	13	17
6-7	123	40.7	40	42	41
8-9	103	34.1	24	43	36
10-11	21	7.0	3	8	10
12-13	9	3.0	0	4	5
14-15	1	0.3	0	1	0
16-17	1	0.3	0	0	1
Total	302	100.0	78	112	112

Mean: 7.39. Standard deviation: 2.00. Standard error of mean: 0.12.

B. Smallpox only					
0-1	0	0	0	0	0
2-3	1	0.4	0	1	0
4-5	43	18.0	12	17	14
6-7	97	40.6	34	28	35
8-9	81	33.9	26	31	24
10-11	16	6.7	3	5	8
12-13	1	0.4	0	0	1
Total	239	100.0	75	82	82

Mean: 7.09. Standard deviation: 1.74. Standard error of mean: 0.11.

t (A and B) = 1.86. $0.10 > P > 0.05$.

tion of reactions by size and in mean size (see Table 10).

Local reaction to smallpox revaccination

As mentioned above, all infants in the "simultaneous" group and in the "smallpox only" group were given smallpox revaccination at 12 weeks at the same time as tuberculin testing. However, for the evaluation of the result of smallpox revaccination, they had to be divided into two categories: those who responded successfully to the initial vaccination im-

mediately after birth, and those who failed to develop a primary lesion at that time.

Those with previous successful take. Table 11 shows the number and percentage of infants in whom vesiculation was present, and, for those with vesiculation, the number of lesions after seven days in response to revaccination at 12 weeks in the "simultaneous" group and in the "smallpox only" group. It is readily seen that only six in the first group and three in the second had vesiculation after

TABLE 8
DISTRIBUTION OF BCG LESIONS BY SIZE AT 4 WEEKS

Size of lesion (diameter in mm)	Simultaneous group				BCG only group (3)	
	Total (1)		Those with take at initial smallpox vaccination (2)			
	No.	%	No.	%	No.	%
0-1	0	0	0	0	1	0.4
2-3	48	19.8	36	19.7	44	18.0
4-5	138	57.2	108	59.1	134	54.7
6-7	32	13.2	22	12.0	31	12.6
8-9	9	3.7	5	2.7	17	6.9
10-11	10	4.1	7	3.8	10	4.1
12-13	3	1.2	3	1.6	6	2.5
14-15	2	0.8	2	1.1	1	0.4
16-17	0	0	0	0	0	0
18-19	0	0	0	0	1	0.4
Total	242	100.0	183	100.0	245	100.0
Mean	4.95		4.93		5.19	
Standard deviation	2.20		2.25		2.48	
Standard error of the mean	0.14		0.17		0.16	

t (1 and 3) = 1.17; $0.30 > P > 0.20$.

t (2 and 3) = 1.16; $0.30 > P > 0.20$.

TABLE 9
DISTRIBUTION OF BCG LESIONS BY SIZE AT 12 WEEKS

Size of lesion (diameter in mm)	Simultaneous group (1)		BCG only group (2)	
	No.	%	No.	%
0-1	0	0	0	0
2-3	58	32.6	54	31.4
4-5	110	61.8	107	62.2
6-7	10	5.6	11	6.4
Total	178	100.0	172	100.0
Mean	3.96		4.00	
Standard deviation	1.11		1.13	
Standard error of the mean	0.08		0.09	

t (1 and 2) = 0.33. $0.80 > P > 0.70$.

seven days. However, there were more with vesiculation and a considerable number of infants with erythema at three days in both groups. The distribution of the reactions by size, i.e., vesiculation and erythema, at three days and seven days, is shown in Table 12 for the "simultaneous" group and "smallpox only" group separately. It is seen that about half the vesiculation and almost all the erythema had disappeared by the seventh day, and that the distribution of vesiculation and erythema by size at three and seven days for both groups shows almost the same pattern: when vesiculation persisted from the third until the seventh day inclusive, it increased in size.

Those with no previous take. Although they, too, are considered revaccination lesions in this category of infants, the lesions developed at this time, in fact, represent a primary take. Table 13 shows the number and percentage of infants with vesiculation, together with the number of lesions at seven days for the "simultaneous" group and "smallpox only" group. Eighteen infants in the "simultaneous" group and eight in the "smallpox only" group, or 32.7% and 23.5% respectively, again failed to show a primary take at the seventh day. However, the difference between the groups is not statistically significant. As seen in Table 14 the distribution, by size, of vesiculation at three days and seven days is obviously different from that shown in Table 12; by the seventh day not only had the number of vesiculations increased but so also had the size of the vesiculation, and this was true of both groups. The erythema, if any, had disappeared by the seventh day in both groups.

Initial smallpox vaccination for the "BCG only" group

All the infants in the "BCG only" group received their initial smallpox vaccination at 12 weeks, and the results were observed seven days after vaccination. As seen in Table 15, the rate of no take at this time was 30.8%, slightly higher than the no-take rates in the other two groups at the initial vaccination immediately after birth, which were 24.3% and 21.5% respectively.

Table 16 shows the distribution by size of positive vaccination lesions in this group; the pattern is fairly similar to that of the two groups that had received smallpox vaccination shortly after birth (see Table 7). However, the mean size of 8.63 mm is slightly larger than that found in the other two groups, which was 7.39 mm and 7.09 mm respectively.

TABLE 10
DISTRIBUTION OF TUBERCULIN REACTIONS BY SIZE AT 12 WEEKS ^a

Size of reaction (diameter in mm)	Simultaneous group				Smallpox only group		BCG only group (3)	
	Total (1)		Those with take at initial smallpox vaccination (2)					
	No.	%	No.	%	No.	%	No.	%
0-1	0	0	0	0	83	47.4	2	0.9
2-3	3	1.5	3	1.8	62	35.4	3	1.4
4-5	13	5.8	11	6.5	22	12.6	6	2.8
6-7	12	5.3	9	5.3	7	4.0	11	5.1
8-9	15	6.7	11	6.5	1	0.6	14	6.5
10-11	40	17.8	27	15.9	0	0	39	18.2
12-13	65	28.9	52	30.6	0	0	54	25.3
14-15	52	23.1	38	22.3	0	0	58	27.2
16-17	18	8.0	12	7.0	0	0	21	9.8
18-19	6	2.7	6	3.5	0	0	6	2.8
20-21	1	0.4	1	0.6	0	0	0	0
Total	225	100.0	170	100.0	175	100.0	214	100.0
Mean	11.94		11.90		12.19			
Standard deviation	3.45		3.59		3.43			
Standard error of mean	0.23		0.27		0.23			

t (1 and 3) = 0.76; $0.50 > P > 0.40$. t (2 and 3) = 0.82; $0.50 > P > 0.40$.

^a Read 72 hours after testing.

Glandular enlargement

Table 17 shows the distribution by size of the right axillary lymph glands at one week, for the "simultaneous" group and the "smallpox only" group. There were respectively 20 and 14 infants in the two groups with enlargements of 10 mm or

more of the axillary lymph glands on the right side, i.e., on the smallpox-vaccinated side, but, on the whole, the distribution by size in both groups was almost the same.

As seen in Table 18, only one in the "simultaneous" group and four in the "BCG only" group

TABLE 11
RESULTS OF SMALLPOX REVACCINATION AT 7 DAYS FOR INFANTS WITH PREVIOUS TAKE

Group	Total		Vesiculation present				Vesiculation absent	
			One lesion		Two lesions			
	No.	%	No.	%	No.	%	No.	%
Simultaneous	170	100.0	6	3.5	0	0	164	96.5
Smallpox only	141	100.0	3	2.1	0	0	138	97.9

$\chi^2 = 0.42$. $P > 0.637$.

TABLE 12
DISTRIBUTION OF SMALLPOX REVACCINATION LESIONS BY SIZE AT 3 AND 7 DAYS
FOR INFANTS WITH PREVIOUS SUCCESSFUL TAKE

Size of lesion (diameter in mm)	Vesiculation				Erythema			
	3 days		7 days		3 days		7 days	
	No.	%	No.	%	No.	%	No.	%
A. Simultaneous group								
0-1	0	0	0	0	1	1.1	0	0
2-3	6	46.2	0	0	43	48.9	1	50.0
4-5	6	46.2	1	16.7	42	47.7	0	0
6-7	1	7.6	3	50.0	2	2.3	1	50.0
8-9	0	0	2	33.3	0	0	0	0
10-11	0	0	0	0	0	0	0	0
12-13	0	0	0	0	0	0	0	0
14-15	0	0	0	0	0	0	0	0
16-17	0	0	0	0	0	0	0	0
Total	13	100.0	6	100.0	88	100.0	2	100.0
B. Smallpox only group								
0-1	0	0	0	0	0	0	0	0
2-3	3	50.0	0	0	42	60.0	1	50.0
4-5	3	50.0	1	33.3	25	35.7	1	50.0
6-7	0	0	2	66.7	3	4.3	0	0
8-9	0	0	0	0	0	0	0	0
10-11	0	0	0	0	0	0	0	0
12-13	0	0	0	0	0	0	0	0
14-15	0	0	0	0	0	0	0	0
16-17	0	0	0	0	0	0	0	0
Total	6	100.0	3	100.0	70	100.0	2	100.0

TABLE 13
RESULTS OF SMALLPOX REVACCINATION AT 7 DAYS FOR INFANTS
WITH NO PREVIOUS TAKE

Group	Total number of infants		Vesiculation present				Vesiculation absent	
			One lesion		Two lesions			
	No.	%	No.	%	No.	%	No.	%
Simultaneous	55	100.0	21	38.2	16	29.1	18	32.7
Smallpox only	34	100.0	16	47.1	10	29.4	8	23.5

$$\chi^2 = 1.01. \quad 0.607 > P > 0.368.$$

TABLE 14
DISTRIBUTION OF SMALLPOX REVACCINATION LESIONS BY SIZE AT 3 AND 7 DAYS
FOR INFANTS WITH NO PREVIOUS TAKE

Size of lesion (diameter in mm)	Vesiculation				Erythema			
	3 days		7 days		3 days		7 days	
	No.	%	No.	%	No.	%	No.	%
A. Simultaneous group								
0-1	1	3.4	0	0	0	0	0	0
2-3	13	44.8	0	0	5	55.6	0	0
4-5	14	48.4	0	0	4	44.4	0	0
6-7	1	3.4	6	16.2	0	0	0	0
8-9	0	0	15	40.6	0	0	0	0
10-11	0	0	9	24.3	0	0	0	0
12-13	0	0	3	8.1	0	0	0	0
14-15	0	0	3	8.1	0	0	0	0
16-17	0	0	1	2.7	0	0	0	0
Total	29	100.0	37	100.0	9	100.0	0	0

Mean: 9.69. Standard deviation: 2.51. Standard error of mean: 0.41.

B. Smallpox only group								
0-1	0	0	0	0	0	0	0	0
2-3	11	50.0	0	0	4	80.0	0	0
4-5	10	45.5	2	7.7	1	20.0	0	0
6-7	1	4.5	7	26.9	0	0	0	0
8-9	0	0	13	50.0	0	0	0	0
10-11	0	0	3	11.5	0	0	0	0
12-13	0	0	1	3.9	0	0	0	0
14-15	0	0	0	0	0	0	0	0
16-17	0	0	0	0	0	0	0	0
Total	22	100.0	26	100.0	5	100.0	0	0

Mean: 8.04. Standard deviation: 1.82. Standard error of mean: 0.36.

t (A and B) = 3.02. $0.01 > P > 0.001$.

showed enlargements of 10 mm or more in the axillary glands on the left side, i.e., on the BCG-vaccinated side, at four weeks; none of these supplicated subsequently. Again there was no significant difference between these two groups of infants in the distribution by size of lymph glands.

At the fifth visit, i.e., seven days after the tuberculin testing and smallpox vaccination of all infants

in the three groups, the axillary lymph glands were palpated. As seen in Table 19, no child in any of the three groups showed enlargement of the left axillary glands. Only a small number of infants in the "simultaneous" group and the "smallpox only" group showed enlargement on the right side while, in the "BCG only" group, relatively more infants showed enlargement because of recent smallpox vaccination on the same side.

TABLE 15
RESULTS OF SMALLPOX VACCINATION AT ONE WEEK
FOR BCG ONLY GROUP

Total number of infants		Vesiculation present				Vesiculation absent	
		One lesion		Two lesions			
No.	%	No.	%	No.	%	No.	%
214	100.0	75	35.1	73	34.1	66	30.8

TABLE 16
DISTRIBUTION OF SMALLPOX VACCINATION LESIONS,
BY SIZE AT ONE WEEK FOR BCG ONLY GROUP

Size of lesion (diameter in mm)	Total number of lesions		One lesion	Two lesions	
	No.	%		Lesion I	Lesion II
0-1	0	0	0	0	0
2-3	1	0.5	1	0	0
4-5	20	9.1	12	6	2
6-7	65	29.4	28	12	25
8-9	58	26.2	17	23	18
10-11	44	19.9	8	15	21
12-13	21	9.5	8	10	3
14-15	12	5.4	1	7	4
16-17	0	0	0	0	0
Total	221	100.0	75	73	73

Mean: 8.63. Standard deviation: 2.66. SE of mean: 0.18.

TABLE 17
DISTRIBUTION OF RIGHT AXILLARY LYMPH GLANDS,
BY SIZE AT 1 WEEK

Size of gland (diameter in mm)	Simultaneous		Smallpox only	
	No.	%	No.	%
0	169	66.8	148	74.0
1-9	62	25.1	38	19.0
10 or more	20	8.1	14	7.0
Total	251	100.0	200	100.0

$$\chi^2 = 2.47. \quad 0.368 > P > 0.223.$$

TABLE 18
DISTRIBUTION OF LEFT AXILLARY LYMPH GLANDS,
BY SIZE AT 4 WEEKS

Size of gland (diameter in mm)	Simultaneous		BCG only	
	No.	%	No.	%
0	218	90.1	218	89.0
1-9	23	9.5	23	9.4
10 or more	1	0.4	4	1.6
Total	242	100.0	245	100.0

$$\chi^2 = 1.77. \quad 0.607 > P > 0.368.$$

TABLE 19
DISTRIBUTION OF AXILLARY LYMPH GLANDS, BY SIZE AT 12 WEEKS

Size (diameter in mm)	Simultaneous				Smallpox only				BCG only			
	Right		Left		Right		Left		Right		Left	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
0	206	91.6	225	100.0	162	92.6	175	100.0	166	77.6	214	100.0
1-9	16	7.1	0	0	11	6.3	0	0	40	18.7	0	0
10 and over	3	1.3	0	0	2	1.1	0	0	8	3.7	0	0
Total	225	100.0	225	100.0	175	100.0	175	100.0	214	100.0	214	100.0

Complications

Three cases of suspected generalized vaccinia, two cases of severe smallpox vaccination lesions, and one case of local secondary vaccinia occurred during the trial.

Of the three cases of suspected generalized vaccinia, two were in the "simultaneous" group and one in the "smallpox only" group. One boy of the former group had a rather strong local reaction with large and deep smallpox vaccination lesions. Ten days after vaccination, multiple small pustules appeared in the area surrounding the smallpox vaccination lesions. Later, he developed one small pustule on the forehead and three on the right sole (about 2×2 mm in size). A moderate degree of fever lasted for two days. The vaccination lesions healed in three, the other pustules in two, weeks. The boy's twin brother, who had a similar reaction but showed only multiple small pustules around the vaccination lesions, was considered to be a case of local secondary vaccinia. The BCG lesions were normal in both twins and neither had axillary lymph gland enlargements.

Another baby boy in the "simultaneous" group developed generalized pustules 11 days after vaccination and received an injection at a private paediatric clinic. When the nurse visited his home on notification by his father three weeks after vaccination, she found a number of scabs over the body surface and at the site where pustules were said to have been present. The BCG lesion showed an induration 3 mm in diameter. No glandular enlargement was felt in the left axillary region, but a gland 10 mm in size was found on the right side on the visits at one week and four weeks.

The third case of suspected generalized vaccinia occurred in the "smallpox only" group. On the home visit at one week, the two smallpox vaccination lesions appeared as vesicles, both 9 mm in diameter. There was an enlarged lymph gland less than 10 mm in diameter in the right axillary region. When this child was brought to the clinic 16 days after vaccination, his father said that multiple small pustules had appeared all over the body surface. These had become confluent later on and had formed scabs. There were extensive confluent scabs on the head. The scabs on both shoulders and arms had fallen off and the scars looked like those of burns. After three days, when the nurse visited the child's home, she found about 15 fresh vesicles (1×2 mm in size) on both forearms.

These infants made an uneventful recovery from

their complications. All had been vaccinated on the third or fourth day after birth, and their birth-weights had all been greater than 3000 g.

One of the two cases of severe local reaction to smallpox vaccination occurred in the "simultaneous" group and the other in the "smallpox only" group. The former was a boy whose birth-weight was 3900 g and who was vaccinated 48 hours after birth. The two smallpox vaccination lesions at one week showed vesiculation of 5 mm and 6 mm in diameter respectively. In the right axillary region there was an enlarged lymph gland slightly more than 10 mm in size. On notification by the parents, a further home visit was paid three weeks after the vaccination. It was found that the two vaccination lesions had become large and deep ulcers, 10 mm and 14 mm in diameter respectively, surrounded by a large area of erythema. The right axillary glands remained about the same size. However, on the visit at one week, the ulcer had already healed, leaving fresh scars of about the same size. The BCG lesion followed a normal course, with induration 5 mm in diameter at four weeks.

The second case was that of a boy who received smallpox vaccine alone three days after birth. His birth-weight was 3350 g. At the visit at one week, the two local lesions were at the stage of vesiculation, both 10 mm in diameter, with an enlarged lymph gland of 10 mm in the right axillary region. Sixteen days after vaccination, the boy was brought to the clinic with large and deep ulcers, 25 mm and 17 mm in diameter respectively. It was said that 10 days after the vaccination small areas of erythema had appeared all over the body surface and then subsided quickly. Erythromycin powder was applied locally for five days and the lesions became clean and healed gradually.

Two infants died during the trial. One death occurred in the "simultaneous" group and one in the "smallpox only" group. Both were premature babies, with birth-weights of 2600 g and 2640 g respectively. One infant died seven, and the other 10 days, after birth, or five and eight days after vaccination. According to the attending physicians, both infants had weak sucking power, which was further weakened after vaccination and, although they were put into the incubator, their condition did not improve. Both developed smallpox vaccination lesions of ordinary size. In the opinion of the attending physicians, death was due to the low vitality of the babies resulting from their being born prematurely.

DISCUSSION

Since Møller's advocacy (1957) of a controlled study of allergic reactions and local responses induced by simultaneous as compared with independent BCG and smallpox vaccination, four such studies have been reported in eastern Asia, two from Japan, one from Hong Kong and one from Taiwan. Of these four, two were concerned with animal experiments while the other dealt with humans.

Shimokawa (1960) has reported that "the results of simultaneous vaccination of infants under the age of one-and-a-half years were not different from those obtained from single vaccinations in terms of the take rate, the size of various vesicles for smallpox vaccination, as well as in terms of the tuberculin reactions and the local lesions for BCG vaccination". He also observed that there was no marked difference between simultaneous vaccination and combined vaccination in respect of the above-mentioned particulars.

However, the single vaccinations with smallpox vaccine constituting a control to his "simultaneous" or "combined vaccination" groups were, in fact, not given concurrently and, in the absence of a control group, Shimokawa compared the level of tuberculin allergy obtained in his study groups with those reported by the Japan BCG Research Council some five years earlier.

Although the study population and the design of the experiments in the study conducted in Hong Kong by Moodie & Cheng (1962) were rather similar to those of the present study, there was a difference in the time schedule for vaccination and follow-up schemes. The method of BCG vaccination was also different: while the multiple-puncture method was employed in the Hong Kong study, intradermal vaccination was used in the present study, the latter being the standard method in UNICEF/WHO-assisted BCG programmes. The most significant difference in design between the two studies was that while provision for smallpox revaccination for the purpose of assessing the degree of immunity conferred by the initial vaccination was made in the present study, no such provision was made in the Hong Kong study.

When the results of both studies are compared, it is apparent that there is a slight difference in the take rate of the groups vaccinated against smallpox and the level of post-vaccination tuberculin allergy in the BCG-vaccinated groups. Nevertheless, the differences between the "simultaneous" group and the single vaccination groups are not great in either study.

TABLE 20
TAKE RESPONSE TO SMALLPOX VACCINATION
BY MONTH AND TYPE OF VACCINATION

Month	Type of vaccination	Percentage distribution by number of lesions at 7 days			
		Total	None	1	2
A. Vaccination at birth					
May	Simultaneous	100.0	14.3	34.5	51.2
	Smallpox only	100.0	16.9	35.2	47.9
June	Simultaneous	100.0	15.3	22.2	62.5
	Smallpox only	100.0	15.9	40.6	43.5
July	Simultaneous	100.0	40.0	34.7	25.3
	Smallpox only	100.0	33.3	36.7	30.0
B. Vaccination at 12 months					
July	BCG only	100.0	35.6	42.4	22.0
August	BCG only	100.0	40.6	28.1	31.3
September	BCG only	100.0	18.2	33.8	48.0

The no-take rates are comparatively high in the present study: 24.3% and 21.5% for the "simultaneous" vaccination group and the "smallpox only" group, as compared with 8.0% and 6.4% in the Hong Kong study. To avoid batch-to-batch variations in the potency of smallpox vaccines, only one single batch of smallpox vaccine, Batch No. 292-B, was used throughout the present study. The capillary tubes containing the vaccine were kept in a refrigerator at 8°C or below and only taken out for use before work in the morning. As it was suspected that a decrease in potency might occur during storage, unused capillary tubes were returned to the laboratory at the end of the study, i.e., about six months after filling. The result of a potency test on rabbits revealed no evidence of any decrease in potency.

However, the break-down of the no-take rate by month of vaccination shows that it was especially high in July, compared with May and June (see Table 20). Furthermore, the break-down by month of the no-take rate for infants in the "BCG only" group who were initially vaccinated at 12 weeks reveals that it was highest in August and lowest in September. Such a change in the no-take rate suggests that the potency of the smallpox vaccine may have decreased during the hottest months in Taiwan, i.e., July and August, because, during

working hours, the vaccine was exposed to room temperatures that were usually above 32°C.

Inquiries regarding the history of smallpox vaccination in the mothers disclosed that none of them had been revaccinated during pregnancy, so that it is unlikely that immunity transmitted from the mothers as a result of such vaccination was the cause of the poor take-response in these groups. Furthermore, as the same nurse carried out all vaccinations throughout the study, it is unlikely that there were variations in technique.

Although the take-response was generally poor and subject to monthly fluctuations, at no time was the difference between the "simultaneous" group and the "smallpox only" group great enough to permit the conclusion that BCG vaccination given simultaneously exerted any effect on the result of smallpox vaccination.

In this analysis, the results of revaccination response in infants with a previous successful take have been separated from those with no previous take (Tables 11-14). Although two observations were made—one at three days and the other at seven days—it was very difficult to express the percentages of vaccinoid, immediate, and primary reactions; therefore these have been expressed simply by the number of various types of local reaction at three days and seven days. As seen in Tables 11 and 12 very few children showed vesiculation, and the great majority showed either local reaction with erythema or no reaction at all. There is no statistically significant difference between the two groups in the percentage distribution of the various types of reaction. Hence it may be concluded that the degree of immunity conferred by the initial vaccination at birth was almost the same, irrespective of whether BCG vaccination was given simultaneously.

Table 13 shows that 18 out of 55 (32.7%) in the "simultaneous" group and eight out of 34 (23.5%) in the "smallpox only" group who failed to show take-response to the initial vaccination failed again at revaccination after 12 weeks. However, the difference between the two groups is not statistically significant. The size of vesiculation at seven days is comparable with that seen in children with a successful take following initial vaccination at birth.

The no-take rate of infants in the "BCG only" group, who received initial vaccination at 12 weeks, was 30.8%, which is slightly higher than that observed in infants in the two groups who had received initial vaccination at birth, their rates being 24.3% and 21.5% respectively. Moodie & Cheng

(1962) also observed a statistically significant increase in the percentage of non-reactors when smallpox vaccination was given three weeks after, and BCG vaccination at, birth (Group Y, 17.1%).

They stated that this could simply have been due to the fact that the smallpox vaccination was given at the age of three weeks. On the other hand, it is possible that the preceding BCG vaccination had some effect on smallpox reactions. In the absence of a control group in which the infants receive no vaccination at all at birth and are vaccinated against smallpox later, at the same time as the "BCG only" group, no one can explain the real cause of such an increase of non-reactors. However, variations in the potency of batches of smallpox vaccine cannot be ignored.

There is, at present, no direct means of measuring the degree of resistance to tuberculosis conferred by BCG vaccination in humans. However, as Møller (1957) pointed out, "post-vaccination allergy can be accepted as the best available indirect evidence of immunity. The studies carried out so far have demonstrated that no very gross difference in either complication or conversion rate can be expected". He rightly pointed out that "it is not enough to know that vaccination resulted in so and so many per cent. 'positives'. We want to know the total distribution of size of reactions, their mean and their variance". Moreover, he stressed the importance of criteria for the dose and technique employed, and of the "blind" reading of reactions. The design and analysis of the present study meet all these requirements.

Before this study was undertaken, two preliminary studies had been carried out in order to answer the following two questions:

(1) What is the optimum dose of PPD RT 23 with Tween 80 for post-vaccination tuberculin-testing in newborn infants?

(2) Does smallpox vaccination or revaccination given simultaneously with the tuberculin test affect the development of tuberculin sensitivity?

Regarding (1) it was our opinion that 1 TU of PPD RT 23 with Tween 80 is not adequate for use in post-vaccination tuberculin testing, although not much difficulty had been encountered in the testing of unvaccinated children. Since the present study is concerned mainly with the difference in levels of tuberculin allergy between the three vaccination groups, it was decided to use a dose of this particular batch of PPD strong enough to demonstrate even

the slightest difference in the level of tuberculin allergy between different groups. Therefore, a preliminary study¹ was conducted among infants 12 weeks after BCG vaccination at birth. The results can be summarized as follows:

Strength	No. of infants	Mean size	Standard deviation	Remarks
1 TU	36	7.3 mm	3.2 mm	Each dose injected into a different arm of the same infant
5 TU	36	8.7 mm	3.6 mm	

It was thought that there was a good correlation between the level of tuberculin sensitivity and the strength of PPD, and that 5 TU of PPD RT 23 with Tween 80 seemed to be the better of the two for post-vaccination tuberculin testing among infants. Hence it was decided to use 5 TU in this study instead of 1 TU, which is the standard dose in field operations.

To answer the second question, the co-operation of the Taipei Public Health Teaching and Demonstration Centre was enlisted, and a study (Lee, 1962) was conducted among third-grade pupils in a primary school in Taipei City. Five hundred and three pupils were divided into two random groups: one was given a tuberculin test only, while the other received the tuberculin test simultaneously with smallpox vaccination. It was found that the groups had a "positive" rate of 60.2% and 56.5% respectively and a mean size of induration of 11.9 mm and 11.2 mm. The distribution of reaction sizes was rather similar. Therefore, it was concluded that smallpox vaccination does not have a significant effect on the tuberculin test given simultaneously. It was then decided that smallpox vaccination or revaccination should be given simultaneously with the tuberculin test at 12 weeks to all infants in the three groups. This not only facilitated follow-up examinations in this study, but will help in the future application of simultaneous vaccination, because if the tuberculin test can be given at the same time as smallpox vaccination, infants and their mothers need not pay additional visits.

The results of tuberculin tests (see Table 10 and the figure) in the "simultaneous" and in the "BCG only" group have very similar patterns. Both show a normal distribution with no statistically significant difference in the mean size of induration. On the other hand, the "smallpox only" group shows an entirely different type of distribution, with a peak at

0 mm and a decrease toward the larger sizes. This is the natural status of tuberculin sensitivity in infants of this age in Taiwan. Apparently, BCG vaccination is responsible for the difference between the former two groups and the latter group; furthermore, smallpox vaccination at birth does not influence the development of tuberculin sensitivity as a result of simultaneous BCG vaccination.

In the Hong Kong study (Moodie & Cheng, 1962), a Mantoux test was given to Groups X and Y (Group X: BCG and smallpox vaccination within three days of birth; Group Y: BCG vaccination within three days of birth and smallpox vaccination three weeks later). As a result, conversion rates of 94.8% and 96.5% (an area of induration 5 mm or more being considered as positive) and mean sizes of 8.76 mm and 8.51 mm respectively were obtained. However, Group Z (the "smallpox only" group) was not tuberculin tested, so that no control is available for comparing the results of tuberculin tests in the BCG-vaccinated groups and the group without BCG vaccination.

In our experience, enlargement of the local axillary lymph glands following BCG vaccination of newborn infants is negligible if vaccination is given carefully and the usual dose is administered by the standard technique. In the present study, no subject showed an enlargement of 10 mm or more of the left axillary glands at 12 weeks; nor was there serious enlargement of the right axillary lymph glands following smallpox vaccination, although there were different degrees of minor enlargement.

While there were no complications after BCG vaccination, there were some in the smallpox-vaccinated groups.

There were three cases of suspected generalized vaccinia, two cases of large local smallpox vaccination lesions, and one case of local secondary vaccinia. It should be noted that a twin brother of the infant who had local secondary vaccination lesions also showed a few pustules on the forehead and right foot, and is included among the three cases of suspected generalized vaccinia. Twins are usually considered to have a fairly similar constitutional make-up, and this may have had some bearing on the significance of the observations. Of the three cases of suspected generalized vaccinia, two belonged to the "simultaneous" group and one to the "smallpox only" group. As has been stated, none of these three cases was very severe, and all healed completely in a matter of a few weeks. One case in

¹ Tuberculosis Control Program in Taiwan (1962) *Monthly report*. April (unpublished document).

each of the two groups developed large smallpox vaccination lesions.

No case of post-vaccinal encephalitis was observed in the present series, but this is so rare a complication that it may very well not occur in a limited study.

Not a single case of complications was reported by Shimokawa (1960) or Moodie & Cheng (1962).

However, the extent to which complications are discovered is necessarily related to the intensity of follow-up and the alertness of research workers. It is thought that the frequency of complications in the present study should not be used as an argument against the field application of simultaneous vaccination.

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RÉSUMÉ

La présente enquête, effectuée à Taipei, avait pour but d'étudier les résultats de la vaccination simultanée par le vaccin antivariolique et par le vaccin BCG chez de très jeunes enfants.

696 nouveau-nés ont été répartis en trois groupes: le premier a reçu simultanément les deux vaccins; le deuxième, le vaccin antivariolique seul, et le troisième, uniquement le BCG. Les vaccinations ont généralement eu lieu entre la 24^e et la 96^e heure après la naissance. L'évolution a été contrôlée au 7^e jour, à la 4^e et à la 12^e semaine. Au cours de ce dernier examen, on a procédé aux épreuves tuberculiques, vacciné contre la variole les enfants du 3^e groupe et revacciné les enfants des groupes 1 et 2.

La réponse à la vaccination variolique a été sensiblement la même dans les groupes 1 et 2, qu'il s'agisse du pourcentage de réactions positives aux primo-vaccinations, du degré d'importance des réactions vaccinales ou de celles succédant à la revaccination.

Lors des contrôles de la 4^e et de la 12^e semaine, aucune différence significative n'est apparue entre les résultats

de la vaccination par le BCG enregistrés dans le groupe 1 et le groupe 3. Les tests à la tuberculine ont montré la même concordance. Dans le groupe vacciné uniquement contre la variole, la réponse au test a été très faible et semblable à celle des enfants de cet âge dans la région. La vaccination antivariolique n'a donc eu aucune influence sur le développement de l'allergie tuberculeuse.

L'auteur relate ensuite l'histoire clinique des complications survenues à la suite de la vaccination antivariolique: trois cas de vaccine généralisée, dont deux dans le groupe 1, deux cas de réactions locales sévères et un cas de lésions vaccinales secondaires. Deux décès de nouveau-nés furent attribués à la prématurité. La vaccination par le BCG n'entraîna aucune complication.

Selon l'auteur, ces résultats démontrent l'absence d'interférence lors de l'administration simultanée du vaccin antivariolique et du BCG, et les complications observées ne doivent pas être invoquées contre l'application de cette double vaccination chez les nouveau-nés.

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