and Ferguson-Smith, 1959) are easily recognized, and are usually indications that megaloblastic changes are present.

The significance of the transitional (intermediate) megaloblast has been the subject of controversy: Dacie and White (1949) and Mollin (1959) believe that transitional megaloblasts are the characteristic marrow reaction where minor deficiencies of vitamin B₁₂ or folic acid are present. Fudenberg and Estren (1958) argue that these cells occur because of a superadded deficiency of iron where a deficiency of folic acid or vitamin B₁₂ already exists. With both these points of view Kondi and Foy (1959) disagree, as they describe cases of severe anaemia in which only transitional cells can be found, and state that transitional changes are no more frequent in India, where iron deficiency is very common, than in Africa, where iron deficiency is less common. In our experience patients with severe anaemia usually show unequivocal megaloblasts, but this is not always so; conversely, frankly megaloblastic erythropoiesis is sometimes found when the degree of anaemia is slight. Furthermore, we have found transitional-type erythropoiesis where there is a sufficiency of iron and also where a deficiency exists. The "exaggerated macrocytic response" to parenteral iron described by Scott (1954) suggests that when a deficiency of iron in addition to a deficiency of folic acid exists, a macrocytic blood picture appears only when the iron deficiency is being corrected.

Megaloblastic erythropoiesis in pregnancy is probably due to deficiency of folic acid, and there is a growing volume of opinion that folic acid should be given routinely to all cases of anaemia of pregnancy (Lowenstein *et al.*, 1955; Francis and Scott, 1959; Baines, 1959), though opinions vary regarding what haemoglobin level constitutes anaemia. As many of the cases in this series showed only slight anaemia and were detected only just before or just after delivery, the anaemia might have undergone spontaneous remission. This is not always so, as cases of severe megaloblastic anaemia are sometimes not detected until the puerperium.

In this series there have been many cases which showed obstetrical complications other than anaemia; it may be that these complications are aggravated by folic-acid deficiency.

Badenoch *et al.* (1955) suggest that toxæmia may cause anaemia by reducing the ability of the mother's intestine to absorb haemopoietic factors. Another view is that anaemia may be a factor in the development of toxæmia. It might therefore be expected that mothers who develop pre-eclamptic toxæmia would be more anaemic than those who do not. In this series there has been no difference in the average haemoglobin level of the two groups, yet the association between megaloblastic erythropoiesis and toxæmia is high and seems to merit further study.

Summary

In an attempt to find the incidence of megaloblastic erythropoiesis in a maternity unit routine study of blood smears was carried out on antenatal patients showing anaemia and on all patients admitted to the maternity hospital. Marrow biopsies were carried out on all patients showing abnormality of the blood films other than cases of hypochromic anaemia. The incidence of megaloblastic erythropoiesis was found to be 1 case

in 26.6 deliveries and 1 case in 4.2 twin deliveries. The condition exists frequently without significant anaemia.

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MULTIPLE-PUNCTURE VACCINATION IN THE NEWBORN WITH FREEZE-DRIED B.C.G. VACCINE

BY

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B.C.G. vaccination has been available in Great Britain for 10 years, and its use in certain groups in the community is now generally accepted. During this period 17,500 newborn infants have been vaccinated with B.C.G. in the University Department of Child Health at St. Mary's Hospital, Manchester. At the outset fresh Danish vaccine was used almost entirely, except for a small amount of freeze-dried vaccine prepared at the Pasteur Institute in Paris, which was kept in readiness as a standby in case the fresh supply should at any time fail. In 1955 a British freeze-dried vaccine was introduced into our immunization scheme, and has now replaced liquid vaccines. In doses of 100,000 to 400,000 viable organisms, given intradermally, it has been found to be effective and free from complications.

Intradermal vaccination will always require a high degree of technical skill, and, if too large a dose is given, or the injection made too deeply, glandular complications or local cold-abscess formation are likely. As the call for B.C.G. vaccination is increased in the underdeveloped countries, a reliable method of inoculation requiring less medical skill would obviously offer great advantages. With this in mind, an investigation of multiple-puncture vaccination of newborn infants was undertaken; for this a more concentrated freeze-dried B.C.G. vaccine was used. Since March, 1958, 2,221 babies have been inoculated by this method, which is now increasingly effective and has been free from any complications.

The object of this investigation was to assess those factors which affect the allergy produced by the vaccine, and to attempt to adjust them so that the multiple-puncture technique could be as effective as the intradermal.

Method

Each ampoule of the special freeze-dried vaccine contained 25 mg. moist weight of B.C.G., which was resuspended in 0.2, 0.3, or 0.5 ml. of sterile water, giving a resultant concentration of 125, 83, and 50 mg./ml., respectively. The most-concentrated suspension was applied to the skin by means of a stainless-steel spatula, and the weaker suspensions were ejected on to the skin from a tuberculin syringe with a fine needle.

Initially, an ordinary Heaf multiple-puncture apparatus, designed for tuberculin-testing (Heaf, 1951) and firing six needles to a depth of 1 mm. or 2 mm., was used. By rotating the base-plate through 30 degrees and firing again, 12 punctures could be administered.

For 20 punctures, Type A guns at variable pressure, and Type B standard-pressure guns, were used. The Type A Mark II gun was designed with 20 individually replaceable needles and a fixed firing penetration of 2 mm.; the Mark III gun also had replaceable needles, but could be adjusted to fire at 1, 2, or 3 mm., and had a much stronger spring loading. The Type B standard-pressure guns fired a fixed number of needles, either 6 or 20, to a depth of 2 mm.; these guns were designed to fire at a constant pressure to eliminate variation between different operators (Carpenter, 1955).

The guns were sterilized before each session by boiling or autoclaving, and during the session were sterilized between each inoculation by immersing the base-plate and needle tips in a mixture of ether 25%, ethyl alcohol 50%, and water 25%, for not less than 30 seconds and then shaking off the surplus moisture. At the end of a session, in which 20 to 30 individuals were vaccinated, the base-plate and needle tips were wiped on blood-agar plates, which were then cultured. All the resulting cultures were found to be free from contaminants. The spirit-ether mixture had no adverse effect on the B.C.G. vaccine.

All injections were made into the right upper arm in the deltoid region in order to leave the left arm free for other immunization procedures.

The skin was cleaned with ether and a drop of vaccine placed on the skin. The base-plate of the gun was then used to spread the vaccine evenly over the skin and ensure that all the holes in the base-plate were covered with vaccine. With the arm held by the left hand, the gun was fired firmly and evenly with the right. The excess of vaccine was then wiped off with a sterile swab.

Initially, comparisons were made in a small series of cases to assess the importance of the size of the drop of vaccine and the effect of wiping off the surplus and sterilizing the base-plate of the gun in spirit-ether-water mixture. It was found that the size of the drop was immaterial so long as there was sufficient vaccine to spread evenly over the base-plate, and that wiping off the surplus vaccine and the sterilization of the base-plate and needles did not impair the effect of the vaccine.

All the infants were given appointments to return four weeks after vaccination. About one-half to two-thirds of the mothers kept these appointments, and a further 30 to 40% returned at a later date. At this visit the inoculation site was inspected and the Mantoux 100 T.U. test was performed, being read in 48 hours. The lesion at the vaccination site consisted of macules or papules which occasionally scaled and sometimes became confluent. In the small number of cases in which lesions were pustular, ulceration never occurred. The

resultant scars were almost invisible, and no case of axillary abscess or adenitis has been recorded. A further appointment was made for all the children one year after vaccination; at this visit a Mantoux test 10 T.U. was performed, and, if negative, was followed by 100 T.U.

Results

Number of Punctures and Depth of Penetration.—At the outset the ordinary Heaf multiple-puncture apparatus with six needles, adjustable to fire at a depth of 1 or 2 mm., as devised for tuberculin-testing, was used. The first vaccinations were performed at a depth of 1 mm., the gun being fired once, and in some cases rotated and fired again to produce 12 punctures. For the 20 punctures special models of the guns were made. The results of these investigations are shown in Table I.

TABLE I.—Effect of Number of Punctures and Depth of Penetration

Depth	No. of Pricks	100 T.U. at 4 Weeks			10 T.U. at 1 Year		
		No. of Cases	Mean Diameter (mm.)	% Pos.	No. of Cases	Mean Diameter (mm.)	% Pos.
1 mm.	6	23	9.7	91	22	14.1	86
	12	100	10.5	90	99	14.2	88
	20	9			12		
2 "	6	36	11.7	94	31	14.2	97
	12	112	12.4	96	149	15.5	97
	20	105	13.4	98	129	16.7	97

Guns, Type A Mk I (1 mm.) and Type A Mk II (2 mm.). Vaccine Batch P.2

It can be seen that allergy produced at four weeks and persisting to one year gradually improves as the number of punctures is increased, and that the depth of penetration has a more significant effect than the number of punctures. With newer guns and modified needles the results at four weeks have been improved still further (see Table III), and it is considered that 20 punctures at 2 mm. with a suitable gun will produce adequate tuberculin sensitivity in newborn infants.

Concentration of Vaccine.—The results of varying the concentrations are shown in Table II. It would appear that increasing the concentration of vaccine above

TABLE II.—Concentration of Vaccine

Batch	Concentration mg./ml.	20 Pricks		6 Pricks	
		No. of Cases	100 T.U. at 4 Weeks (Mean Diameter, mm.)	No. of Cases	100 T.U. at 4 Weeks (Mean Diameter, mm.)
P6	50	54	12.5	—	—
	83	28	12.9	43	12.6
P7	83	68	11.9	23	12.8
	125	96	12.4	53	12.6

Gun used, Type A Mk II. Depth of penetration, 2 mm.

50 mg./ml. does not increase the tuberculin sensitivity produced at four weeks after vaccination. For practical purposes a dilution of 83 mg./ml. is the easiest to use, as it is sufficiently fluid to be delivered from a tuberculin syringe through a fine needle, thus eliminating the need for a spatula or platinum loop, and it is viscous enough not to run off the arm, as the thinner 50 mg./ml. suspension tends to do.

Modification of Guns and Needles.—When it was clear that vaccination was effective at a depth of 2 mm. with 20 punctures, and that the effect was not increased by increasing the strength of the vaccine, other methods of improving the technique were considered. The Type A Mark III guns were made with stronger springs, and the needles were roughened first by grooves and

later by providing tungsten tips. The standard-pressure Type B guns with a needle of larger diameter and shorter bevel were used. The results in Table III show

TABLE III.—Type of Gun and Needles Used

Gun	Needles	6 Punctures at 2 mm.			20 Punctures at 2 mm.		
		100 T.U. at 4 Weeks			100 T.U. at 4 Weeks		
		No. of Cases	Mean Induration (mm.)	% Pos.	No. of Cases	Mean Induration (mm.)	% Pos.
Type A Mk II	Smooth	36	11.7	94	105	13.4	98
" " " III	"	60	13.5	95	14	12.8	100
" " " III	Rough	111	15.3	100	50	14.8	100
" " " III	"				34	14.6	100
" B	(B type) Smooth	61	14.8	100	42	14.8	100

Concentration of vaccine, 83 mg./ml. throughout.

that these modifications have been to some extent effective, and that with either 6 or 20 punctures at 2 mm. the later type gun produces a stronger tuberculin sensitivity at four weeks. It is not yet known whether this improvement will be associated with a longer duration of allergy.

Comparison with Results of Intradermal Vaccination

The tuberculin sensitivity produced by intradermal vaccination is related to the number of viable bacilli introduced. For a multiple-puncture technique to be effective it should produce results similar to those pro-

TABLE IV.—Comparison of Intradermal and Multiple Puncture Vaccination

Method	Dose	100 T.U. at 4 Weeks		10 T.U. at 1 Year	
		Range (mm.)	% Pos.	Range (mm.)	% Pos.
I.D.	1×10^4	8.3	82	14.5	93
M.P.	6 punctures at 1 mm	9.7	91	14.1	86
(Type A Mk I)					
M.P.	12 punctures at 1 mm.	10.5	90	14.2	88
(Type A Mk I)					
M.P.	6 punctures at 2 mm	11.7	94	14.2	97
(Type A Mk II)					
M.P.	12 punctures at 2 mm.	12.4	96	15.5	97
(Type A Mk II)					
M.P.	20 punctures at 2 mm.	13.4	98	16.7	97
(Type A Mk II)					
I.D.	1×10^5	11.3-13.4	96.5	16.4-18.0	98
"	2×10^5	14.6	98.7	18.6	100
"	1×10^6	15.3	98.0	19.3	100
M.P.	6 punctures at 2 mm.	13.5-15.3	95-100	Not yet tested	
(Type A Mk III)					
M.P.	20 punctures at 2 mm.	14.8	100	" " "	
(Type A Mk III)

Comparison of results from intradermal (I.D.) and multiple-puncture (M.P.) vaccination.

duced by the optimal dose injected intradermally (in newborn infants, using British freeze-dried vaccine, this is probably of the order 200,000 to 400,000 viable bacilli per dose). Table IV and the accompanying Graph illustrate the results so far achieved. With the Type A Mark II gun the results of vaccination with 6 punctures at 1 mm., and 6 punctures at 2 mm., are probably inadequate; 12 punctures at 2 mm. show a fall after one year; and 20 punctures at 2 mm. bring results within the range produced by 100,000 bacilli given intradermally. With newer guns (Type A Mark III and Type B standard-pressure type) firing 20 needles, the tuberculin sensitivity produced at four weeks is within the range produced by the intradermal injection of 200,000 to 400,000 bacilli. It remains to be seen whether the tuberculin sensitivity at one year continues to be adequate.

Discussion

Conflicting results with regard both to intradermal and to multiple-puncture vaccination may be attributed to variations in antigenicity and potency of the vaccine and in the technique of application.

Although any B.C.G. vaccine applied by any method may produce tuberculin sensitivity and a degree of protection against tuberculosis in some of the subjects vaccinated, it is desirable that these results should be as effective and long-lasting as possible. Using the British freeze-dried vaccine intradermally, it has been possible to correlate to some degree the clinical effects of vaccination with the dose of viable bacilli administered (Griffiths, and Gaisford 1956; Ungar, 1958; Griffiths, 1960), and similar results have been reported with the Japanese vaccine (Obayashi *et al.*, 1959). With multiple puncture vaccination, on the other hand, it is impossible to make any assessment of the number of viable organisms entering the skin, and it is only possible to assess their practical importance by varying some of the factors involved.

Number of Punctures.—Rosenthal (1957) thought that 18 to 36 punctures were essential and found a correlation between clinical effectiveness and number of punctures. Lorber (1959) also noted the importance of this factor. In the present series the increase in tuberculin sensitivity, particularly immediately after vaccination, produced by increasing the number of punctures from 6 to 20 was much less than one would have expected. However, since duration of allergy seemed to be related rather to the number of punctures, it has been decided that 20 punctures should be the routine procedure for vaccination in the newborn at the present time.

Depth of Penetration.—Lorber (1959) suggests that a depth of 1 mm. is sufficient for vaccination in infancy. From the experience in this series there appears to be no doubt that a penetration of 2 mm. gives better results than 1 mm., particularly in the results of tuberculin testing at a later date. This would seem reasonable in that the thickness of the epidermis in newborn infants is such that one would expect penetration of 2 mm. to be the optimum to reach those layers of the corium with adequate lymphatic communications.

Concentration of Vaccine.—Ejlertsen (1953) and Rosenthal (1957) have shown that improved clinical effects can be expected by increasing the concentration of vaccine. In both these reports the range of concentration was from 5 mg./ml. to 50 mg./ml., well below the concentration studied in this present series. It therefore seems likely that a concentration over 50 mg./ml. is

adequate and that it is almost impossible to get a concentration that will produce glandular complications so common with higher doses of intradermal vaccine. Practical reasons for preferring a dosage of 83 mg./ml. have already been mentioned.

Modification of Guns and Needles.—This is a factor which has received very little attention in previous reports. First attempts at multiple-puncture vaccination (Rosenthal, 1939; Rosenthal and Neiman, 1941) by means of 20 to 30 tangential pricks from a single needle were followed by use of Birkhaug's instrument (Birkhaug, 1947), of which the Heaf (1951) multiple-puncture apparatus would seem to be a simple modification. The present Rosenthal technique (Rosenthal, 1957) utilizes a disk with 36 points and does not depend on any spring pressure. At the moment we are interested in the question of the physical factors involved in firing the gun, and this will be discussed in a later paper; it is quite evident, however, that the technique of multiple-puncture vaccination must be as meticulous as that for the intradermal method even though it calls for less basic skill.

Comparison with Results of Intradermal Inoculation.—Previous reports on the relative efficiency of intradermal and multiple-puncture vaccination have been conflicting. Rosenthal (1939) and Rosenthal and Neiman (1941) consider that multiple puncture vaccination is as effective as intradermal and indeed offers more complete protection against tuberculosis. Ejlersen (1953) takes the opposite view. In the present series the aim has been to improve the technique of multiple-puncture vaccination so that it is, as nearly as possible, as effective as the intradermal vaccination with freeze-dried vaccine.

Summary

A total of 2,221 newborn infants have been inoculated with British freeze-dried B.C.G. vaccine by multiple-puncture vaccination between March, 1959, and April, 1960.

Details of technique are described and significant factors discussed.

The results of multiple-puncture vaccination are compared with those of intradermal vaccination.

It is suggested that, using the British freeze-dried vaccine, optimal results are obtained by firing 20 needles to a depth of 2 mm. through a vaccine concentration of 83 mg./ml. It is hoped that by further improvement in technique the efficiency of multiple-puncture vaccination may be raised to the standard produced by intradermal vaccination.

This work was carried out in the Department of Child Health, St. Mary's Hospitals, Manchester, under the direction of Professor Wilfrid Gaisford, and was partially financed by grants from the Research Grants Committee of the United Manchester Hospitals. The vaccine was supplied by Glaxo Laboratories, Greenford, Middlesex, and I am indebted to Dr. J. Ungar and Dr. P. W. Muggleton for their practical help. Allen and Hanburys Ltd. have supplied the Type A guns; the Type B guns were supplied by Messrs. H. G. East for the British Tuberculosis Association pilot trials, and were lent for use in the newborn.

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OBSERVATIONS ON VACCINATING SCHOOLCHILDREN WITH DANISH FRESH B.C.G.

BY

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In 1954 eight medical officers of health in the Oxford Region adopted a uniform procedure for the testing and B.C.G. vaccination of their schoolchildren. The scheme was in full operation by 1955. In that year, however, the P.P.D. issued for routine Mantoux testing was sub-standard. The findings have therefore been analysed from 1956 onward. The overall conversion rates for that year have already been published (Irvine, 1958). In this report the conversion rates and the local reactions to vaccination are studied in greater detail.

Material and Method

The analysis is based on 8,990 routine B.C.G. vaccinations of 13-year-old schoolchildren in the Oxford Region during the school year 1956-57. Mantoux 10 T.U. tests were given for the pre- and post-vaccination tests in 5,390 children and Heaf multiple-puncture tests in 3,600. The standard preparations of P.P.D. issued by the Ministry of Health were used for each test. Both Mantoux and Heaf tests were read at 2 to 4 days; the criterion of a minimum positive reaction for the Mantoux was an area of induration whose diameter was 6 mm., and for the Heaf test induration at four punctures. Non-reactors were vaccinated intracutaneously by some 50 school medical officers with 0.1 ml. of Danish fresh vaccine (0.75 mg./ml.), using about 40 different batches. The post-vaccination test was carried out between 6 and 19 weeks after vaccination. The local reaction to vaccination was inspected at the time of the post-vaccination test, and the diameter in millimetres of the indurated papule was measured with callipers; all Mantoux reactions were also measured with callipers. Where a vaccination or Mantoux reaction was not circular, the measurement recorded was the mean of the largest and the smallest diameter. None of the children was in contact with known open tuberculosis at the time of vaccination.

Results

Hertzberg (1948) found that sex had no influence on the size of the vaccination reaction or the conversion rate. An initial analysis of the present data provided similar results, and this factor has accordingly been ignored in the study.

Conversion Rate.—This has been analysed according to the number of weeks elapsing since vaccination. The