Dementia has now replaced schizophrenia as the commonest cause of a stay in hospital of more than two years.

The decrease in the number of schizophrenic illnesses becoming chronic may well lead to a fall in the total patient population of 30% over the next 20 years. If, in addition, changes in national policy are effective, mental hospitals may perhaps be reduced to one-quarter of their present size.

I am indebted to the medical superintendents and other staff of 38 hospitals for help in tracing the progress of 124 transferred patients; and to Dr. J. A. Scott, Medical Officer of Health for London, and to the Town Clerk of Lewisham for help in calculating the population at risk.

REFERENCES

- Annual Abstract of Statistics (1958 and 1960). H.M.S.O., London.
Brill, H., and Patton, R. E. (1957). *Amer. J. Psychiat.*, **114**, 509.
— (1959). *Ibid.*, **116**, 495.
Kay, D. (1959). *Proc. roy. Soc. Med.*, **52**, 791.
Kramer, M., and Pollack, E. S. (1958). *Amer. J. publ. Hlth*, **48**, 1003.
Norris, V. (1959). *Mental Illness in London*. Maudsley Monograph.
Roth, M. (1960). *Brit. med. J.*, **1**, 1226.
Shepherd, M. (1957). *A Study of the Major Psychoses in an English County*, Maudsley Monograph. Chapman and Hall, London.

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

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The value of B.C.G. vaccination of schoolchildren in this country in the prevention of tuberculosis has been sufficiently substantiated (Medical Research Council, 1956, 1959; Pollock, 1959; Springett, 1959) for it to require no further advocacy. There still remains, however, the problem of how it can be most effectively, rapidly, and economically administered to children, not only in this country but in those overseas countries with greater problems of tuberculosis control, expanding populations, hot climate, and insufficiency of public health personnel. For these countries a heat-stable freeze-dried vaccine which can be easily and efficiently administered would seem to provide the answer.

A freeze-dried vaccine for intradermal use has been prepared and used in Great Britain for several years, and reports on its efficiency in schoolchildren have been made by the Medical Research Council (1958) and by Alston *et al.* (1958). Although, in the strength issued at the time, the freeze-dried vaccine was slightly less effective than the fresh Danish, a slight increase in the bacterial content has eliminated this difference, and the advantages inherent in a freeze-dried vaccine have led to its widespread use.

Recently a stronger freeze-dried vaccine for multiple-puncture vaccination has been available, and reports on its use in schoolchildren have been published from Cardiff and Pembroke (Griffith, 1959) and from Rotherham (Hopper, 1960).

In February, 1959, a trial of this method of vaccination was initiated in four health divisions in north-east Cheshire. The aim of this trial was (a) to compare the results of multiple-puncture with intradermal vaccination, and (b) to make an attempt to assess some of the factors which influence the efficiency of multiple-puncture vaccination and thus to improve the technique or vaccine to such an extent that the degree of local reaction and allergy produced will compare favourably with those from intradermal vaccination.

First Trial (School Year 1958-9)

The object of this trial was to compare the effects of vaccine strength of 83 mg./ml. given by multiple puncture, using 20 needles at depths of 2 or 3 mm., with that produced by an intradermal vaccine of a strength of 500,000 viable organisms per dose.

Method.—Preliminary testing was performed with 10 T.U. of old tuberculin intradermally in the left forearm. Of 1,099 children tested, 791 had negative reactions (less than 5 mm. induration), and these children were vaccinated. Vaccination either by the intradermal route or by multiple puncture at a depth of 2 or 3 mm. was performed in strict rotation, the three groups being equally rotated. For the intradermal route the skin in the right deltoid region was cleaned with ether, and 0.1 ml. of vaccine, prepared by adding 1 ml. of sterile water to each ampoule of dried vaccine, was given intradermally. For the multiple-puncture

FIG. 1.—Left: Type A gun set to fire 20 needles at a depth of 2 mm. Right: Type B gun set to fire 20 needles at a depth of 2 mm.

method Type A guns fitted with 20 needles which were individually replaceable and which could be adjusted to fire to a depth of 2 or 3 mm. were used (Figs. 1 and 2). The guns were sterilized before each vaccinating session by boiling or autoclaving. The skin over the right deltoid was cleaned with ether, and a drop of vaccine, prepared by adding 0.3 ml. of sterile water to each ampoule containing 25 mg. of B.C.G., was placed on the skin. This was spread by the base-plate of the gun, which was then held firmly on to the arm, the needles being fired to the required depth. The base-plate and needle-tips were then immersed in a solution of equal parts of methylated ether and surgical spirit for 30 seconds. The vaccination sites were inspected six weeks after vaccination and another tuberculin test with 10 T.U. of O.T. was performed. This was read 72 hours later. One year later tuberculin tests with 10 T.U. of O.T. were repeated. The results of these investigations are set out in Table I.

FIG. 2.—Type A gun, showing method of changing needles.

The results of Mantoux-testing have been recorded both as the percentage of positive reactors (5 mm. or more induration) and the mean diameter of induration (including the negative reactors). When the percentage conversion reached 95% or more, minor variations became insignificant, and it then

became necessary to rely on the mean induration to give an indication of differences caused by the various factors investigated.

From the first trial (Table I) the following points became obvious: (a) there was a marked variation between the results produced by the four investigators taking part in the trial; (b) in all four instances the intradermal technique produced greater conversion than

TABLE I.—First Trial (School Year 1958–9). Results of 10 T.U. at 6 Weeks and 1 Year. Comparison of Multiple-puncture with Intradermal Vaccination

Method	Results to 10 T.U.							
	Dr. A		Dr. B		Dr. C		Dr. D	
	Mean Diam. (mm.)	% Pos.	Mean Diam. (mm.)	% Pos.	Mean Diam. (mm.)	% Pos.	Mean Diam. (mm.)	% Pos.
6 Weeks								
Intradermal ..	20.7	100	13.0	99	10.1	94	8.9	100
Multiple puncture, 20/2 mm.	18.8	100	9.6	90	7.7	83	7.0	88
Multiple puncture, 20/3 mm.	18.5	100	9.6	91	8.2	92	8.2	93
1 Year								
Intradermal ..	21.7	100	10.7	84	Not tested			
Multiple puncture, 20/2 mm.	20.1	94	5.2	51				
Multiple puncture, 20/3 mm.	20.2	100	5.0	44				

the multiple puncture; and (c) in none was there any significant difference between the effects of multiple puncture at a depth of 2 mm. compared with a depth of 3 mm.

Second Trial (School Year 1959–60)

It was decided that results of multiple-puncture vaccination were promising enough to make it worth while to try to investigate the factors affecting the efficacy of this method. As the first trial had demonstrated the effect of operator variation, a specific problem was assigned to each worker. By eliminating the variable of operator technique in each group it was possible to study several different problems concurrently.

During this period 2,422 children were tested, and 1,744 children with negative reactions to 10 T.U. were vaccinated. The following factors were assessed: (a) the effect of heat on the vaccine; (b) differences in concentration of vaccine; (c) the effect of roughened needles fitted to the guns; (d) comparison of Type A and Type B guns and effect of variation of pressure; and (e) correlation of intensity of local reaction and tuberculin sensitivity.

TABLE II.—Individual Variations in Multiple-puncture Technique

Operator	Series I. First Trial			Series II. Second Trial		
	10 T.U. at 6 Weeks			10 T.U. at 6 Weeks		
	No. of Cases (2 and 3 mm. Depth Combined)	Mean Induration (mm.)	% Positive	No. of Cases	Mean Induration (mm.)	% Positive
Dr. A	83	18.7	100	A.367 (heat effect) B.389	14.6	97
Dr. B	161	9.6	91	A.360 (heat effect) B.297	18.4	98
Dr. C	414	8.0	87	8.4	74	
Dr. D	133	7.8	91	12.8	95	
Total	791			213	9.1	99
				118	9.1	94
				1,744		

The overall results are shown in Table II. As was to be expected the individual variations were still in evidence, but the degree of conversion six weeks after vaccination in most cases was 95%; in some cases where it was below this figure a reason for this could be found. These individual factors are discussed.

(a) *Effect of Heat on Vaccine.*—In the first trial the Type A guns were sterilized between sessions by boiling or autoclaving and between individual vaccinations by immersion of base-plate and needle-tips in a mixture of ether 25%, ethyl alcohol 50%, and water 25% for 30 seconds. A question was then raised regarding the efficacy of this method of sterilization, and the gun was therefore flamed after 10 firings in addition to the other methods of sterilization used. It was immediately noticed that the results obtained in this second series of tests fell far below those produced in the preliminary trial. Accordingly more guns were obtained and each operator used four guns, flaming them after every 10 cases as before, but allowing a much longer period of cooling for each gun. An improvement in results was immediately noticeable (see Table III).

(b) *Concentration of Vaccine.*—Concentrations of 83 mg./ml. and 125 mg./ml. were prepared by adding 0.3 or 0.2 ml. of sterile water to the dried mass (25 mg.) of vaccine in each ampoule. These were used by Dr. B in alternate cases, using different guns for each concentration. The results of this are shown in Table IV and compared with results from Dr. A in two separate

series (one in the first trial and one in the second), using similar concentrations. No difference could be noted in the tuberculin conversion produced by these two concentrations.

(c) *Use of Rough Needles.*—As it had been suggested that roughening of needles might improve the results of multiple-puncture vaccination it was decided to

TABLE III.—Second Trial. Effect of Heat on Vaccine

Method of Sterilization	Dr. A		Dr. B	
	10 T.U. at 6 Weeks		10 T.U. at 6 Weeks	
	Mean Induration (mm.)	% Positive	Mean Induration (mm.)	% Positive
Boiling or autoclaving before sessions. Spirit/ether mixture between cases. No heat	18.8	100	9.6	90
Boiling or autoclaving before sessions. Spirit/ether mixture between cases. Heat every 10th case	14.9	96	8.4	74
Boiling or autoclaving before sessions. Spirit/ether mixture between cases. Gun cooled after heating	18.2	98	12.8	95

TABLE IV.—Effect of Variation in Concentration of Vaccine

Concentration (mg./ml.)	Dr. A		Dr. B	
	10 T.U. at 6 Weeks		10 T.U. at 6 Weeks	
	Induration (mm.)	% Positive	Induration (mm.)	% Positive
83	First trial		Second trial	
	18.8	100	12.5	95
125	Second trial		Second trial	
	18.2	98	13.0	95

FIG. 3.—Correlation of number of papules in local reaction with result of Mantoux 10 T.U. at 6 weeks. (Drs. A, B, and C.)

investigate this factor. Needles were roughened either by grinding a screw thread at the tips or by tipping with tungsten. There was no difference in the tuberculin conversion produced by different types of needles.

(d) *Type of Gun and Pressure Used.*—(1) For this series Dr. A used two types of gun both firing 20 needles to a depth of 2 mm. Type A, in which the needles were individually replaceable, was hand-fired by the operator; and Type B, in which the needles were not replaceable, was so adjusted that it fired only when a certain pressure was exerted, as described by Carpenter and Stewart (1959) (see Figs. 1 and 2). The pressure exerted on firing the gun was investigated by firing 10 times into a cork on a spring balance, and it was found that the pressures were reasonably consistent, varying between 7 and 9 lb. (3.2 and 4 kg.). The tuberculin sensitivity produced by the two guns showed no significant difference, suggesting that the pressure of firing was sufficient to drive all the needles to the requisite depth. (2) Dr. C used Type A guns, deliberately firing alternately heavily and lightly. The pressure exerted in this way was investigated as before with a cork on a spring balance. The light pressure was approximately 5 lb. (2.3 kg.) and the heavy pressure varied between 7 and 9 lb. (3.2 and 4 kg.). There was no significant difference in the tuberculin sensitivity produced. These results suggest that a pressure between 5 and 9 lb. (2.3 and 4 kg.) was adequate to fire 20 needles 2 mm. into the skin.

(e) *Correlation between Local Reaction and Mantoux Sensitivity Produced.*—On examining the vaccination records of the children it was obvious that in addition to differences in tuberculin sensitivity there was a good deal of variation in the local lesion produced by various

FIG. 4.—Correlation of degree of local reaction with result of tuberculin at 6 weeks. x—x=Dr. A. x---x=Dr. B.

operators. The local tissue reaction to B.C.G. vaccination by multiple puncture varies in degree and in the number of papules produced. The number of papules produced in relation to the number of punctures administered may indicate the degree of accuracy of the vaccinator. In order to see if this was the case the percentage conversion to 10 T.U. at six weeks was plotted against the percentage of cases in which 20 papules were produced and the percentage of cases in which fewer than 15 papules were produced by a 20-needle gun (Fig. 3). The factors influencing the number of papules produced may be the technique of the vaccinator, defects in the gun, or variations in the vaccine. The fact that 20 papules are produced indicates adequate spreading of the vaccine, even application of the gun by the vaccinator, and reasonable condition of the gun. If a few needles are blunted or shortened there will be occasional papules missing. If, however, there are a large number of papules missing this suggests either inadequate spreading of the vaccine or uneven application of the gun and is likely to be related to the operator technique. It is notable that when Dr. B (whose results showed the greatest variability) produced a high percentage of reactions with 20 papules the Mantoux conversion was adequate, whereas there was poor Mantoux conversion in those series in which a higher percentage of reactions of fewer than 15 papules were produced. As the same guns and vaccine were used throughout, it is suggested that the variations were in the technique. With regard to the degree of reaction there is likely to be more individual variation in the readings, but it is possible to compare the percentage positive and the mean induration to 10 T.U. at six weeks with the degree of reaction from individual vaccinators. In all cases a degree of reaction producing pustules, scaling, or confluence was associated with tuberculin conversion equivalent to that produced by intradermal vaccination (see Fig. 4). It is therefore apparent that successful technique in vaccination is extremely important.

Discussion

Although multiple-puncture B.C.G. vaccination is by no means a new method of administration and has been used by Birkhaug (1944) since 1939 and by Rosenthal (1939) since 1937, there is as yet no certain evidence with regard to all the factors necessary to ensure that vaccination by this method should be as effective as possible. In all previous reports (Forssman, 1946; Hertzberg, 1948; Geser, 1954; Griffith, 1959; Rosenthal, 1960) in which multiple-puncture vaccination has been compared with intradermal in groups of children of school age, it has been found that multiple-puncture vaccination is less effective than intradermal. This has also been found to be the case in the present trial. Investigations which will increase the efficiency of multiple-puncture vaccination have not been numerous and certainly more need to be undertaken. Although multiple-puncture vaccination would appear to be a simple and easy method of B.C.G. vaccination it is evident that there is considerable variation between the results of individual operators, and that the technique of this method will need to be carefully considered and assessed and be adhered to very strictly. There is no doubt that the children concerned prefer to be inoculated by the multiple-puncture method, and this may have an effect on the acceptance rate and thus the total number of protected children.

C

It is likely that a certain minimal concentration of vaccine is necessary. It is possible that the concentration of 20 mg./ml. favoured by Forssman (1946) and Geser (1954) was inadequate, and that the concentrations used in this present trial of 83 mg./ml. and 125 mg./ml. produced no appreciable difference in clinical efficacy because both concentrations were above the margin required for maximal effect. Griffith (1959), using the same freeze-dried vaccine as in the present trial, reports successful results with a suspension of 50 mg./ml.; Hopper (1960) found a concentration of 1.8 mg./ml. to be quite inadequate. It is probable that eventually the concentration may need to be measured by the number of viable organisms per ml.

Rosenthal (1957) stated that increasing the number of punctures increases the efficiency of vaccination, and Griffith (1959) suggested that the number of punctures used in schoolchildren should be 40. As the guns in the present trial were fitted with only 20 needles it was decided to use this number of punctures in assessing some of the other factors. In further investigations it may well be advisable to increase the number of punctures to 40. It has been observed in the newborn that an increased number of punctures is effective in prolonging the efficiency of vaccination (Griffiths, 1960).

The importance of the effect of heat on the B.C.G. vaccine has not been previously reported, possibly because heat sterilization is not necessary with Rosenthal's (1957) technique, and is not advocated by Birkhaug (1947). In the present series there was no doubt about the deleterious effect of using a hot gun, and it is possible that this may have been one of the factors causing the discrepancy in results reported from Cardiff and Pembrokeshire by Griffith (1959).

It is also probable that the type of gun used may affect the results. No difference has been noted in the effects produced by 20-needle guns Type A and Type B, but it is possible that further investigations will lead to modifications in the guns to produce improved results.

Hopper (1960) reported that as the needles became blunter through the effects of flaming and clogging with carbon deposits, the clinical results deteriorated. We have found no differences in results using specially roughened needles.

The fact that there was evidence of considerable variation between operators emphasizes the necessity for establishing a reliable technique in the use of multiple-puncture instruments, to ensure adequate penetration of all the needle points.

Summary

2,535 Cheshire schoolchildren were vaccinated with the British freeze-dried B.C.G. by the multiple-puncture technique between February, 1959, and June, 1960.

Multiple-puncture vaccination with freeze-dried B.C.G. was less effective than intradermal vaccination with a less concentrated freeze-dried vaccine.

When guns firing 20 needles at a pressure of 5-9 lb. (2.3 and 4 kg.) were used, there was no significant difference in the clinical effects produced by puncturing to depths of 2 or 3 mm., using vaccine concentrations of 83 mg./ml. or 125 mg./ml., self-firing or operator-fired guns, and rough or smooth needles.

It was found that heat had a deleterious effect on the vaccine, and adequate time for cooling was necessary if this method of sterilization was practised.

Although this is apparently a simple method there was a great deal of operator variation, and further investigations into the technical factors affecting the results of multiple-puncture vaccination need to be carried out in the hope of modifying this technique to make the results comparable with those produced by intradermal vaccination.

We thank Dr. Arnold Brown, Medical Officer of Health for Cheshire County Council, for allowing this work to be carried out, and Professor Wilfrid Gaisford, Department of Child Health, Manchester University, for his encouragement and advice. The vaccine was prepared at, and supplied by, the Glaxo Laboratories, and we are grateful to Dr. J. Ungar and Dr. P. W. Muggleton for their practical help throughout the investigation. The Type A guns and the roughened needles were supplied by Messrs. Allen and Hanburys, of London, and the Type B self-firing gun was supplied for the B.T.A. pilot trial by Messrs. H. G. East, of Oxford. The photographs were taken by Mr. D. F. Boxall.

REFERENCES

- Alston, P., Cowell, J. St. B., and O'Meara, R. A. Q. (1958). *Irish J. med. Sci.*, p. 304.
 Birkhaug, K. (1944). *Acta med. scand.*, **117**, 274.
 — (1947). *Amer. J. clin. Path.*, **17**, 751.
 Carpenter, R. G., and Stewart, C. J. (1959). *Tubercle (Lond.)*, **40**, 251.
 Forssman, O. (1946). *Acta tuberc. scand.*, **20**, 123.
 Geser, A. (1954). *Dis. Chest*, **26**, 62.
 Griffith, A. H. (1959). *Lancet*, **1**, 1170.
 Griffiths, M. I. (1960). *Brit. med. J.*, **2**, 1116.
 Hertzberg, H. (1948). *The Achievement of B.C.G. Vaccination*. Grundt, Oslo.
 Hopper, J. M. H. (1960). *Med. Offr.*, **103**, 311.
 Medical Research Council (1956). *Brit. med. J.*, **1**, 413.
 — (1958). *Ibid.*, **1**, 79.
 — (1959). *Ibid.*, **2**, 379.
 Pollock, T. M. (1959). *Tubercle (Lond.)*, **40**, 399.
 Rosenthal, S. R. (1939). *Amer. Rev. Tuberc.*, **39**, 128.
 — (1957). *B.C.G. Vaccination Against Tuberculosis*. Little, Brown, Boston and Toronto.
 — (1960). *Bull. int. Un. Tuberc.*, Special No. on Methods for the Study of B.C.G. Vaccination, p. 256.
 Springett, V. H. (1959). *Tubercle (Lond.)*, **40**, 432.

Bowdler (1958). Zarafonitis *et al.* (1957) describe the unique sequence of pernicious anaemia, polycythaemia, and acute leukaemia occurring in the same patient.

Case Report

A married woman aged 66 was first seen by one of us (W. R.) in October, 1945. She complained of loss of weight, lethargy, and cramps in the legs. These symptoms had developed gradually over the preceding few months and her memory had deteriorated. Her previous health had been good, her husband stating that she had usually been of good facial colour.

Clinically she was a small, spare woman with slight kyphosis. She did not appear obviously anaemic. No noteworthy physical signs of disease were found apart from a sensation of fullness on palpation of the left hypochondrium. When seen in consultation by Dr. Bellingham Smith a diagnosis of carcinoma of the stomach or colon was considered. Blood examination at that time showed: Hb, 36% (5.3 g./100 ml.), R.B.C., 2,110,000/c.mm.; C.I. 0.9. The red cells showed macrocytosis, and varied in size and shape. A barium-meal examination showed no radiological abnormality of stomach or duodenum, but as the patient was unwilling to be admitted to hospital (an attitude she firmly maintained subsequently) she was treated at home with intramuscular injections of liver extract ("campolon") and oral iron. Such treatment was associated with rapid clinical and haematological improvement (see Chart) and five months later the somewhat surprising result of Hb 135% (20 g./100 ml.) and R.B.C. 7,280,000/c.mm. was reported. Clinically she had become obviously polycythaemic with red face and watering blood-shot eyes. A mass now readily palpable in the left hypochondrium was regarded as an enlarged spleen with a firm edge. The patient had lost all her old symptoms and felt remarkably well.

All treatment was discontinued and she apparently remained well until the autumn of 1948, when her original symptoms returned. In December, 1948, severe macrocytic anaemia was again present: Hb, 44% (6.5 g./100 ml.); R.B.C., 2,000,000/c.mm.; C.I., 1.1. The spleen was larger. Treatment with further parenteral liver extract alone was again followed by prompt improvement with another meteoric rise in Hb and red-cell count. Within three months of restarting liver therapy the Hb was 140% (20.8 g./100 ml.) and R.B.C. 8,500,000/c.mm. She felt well but was very plethoric. The spleen was now palpable fully a handbreadth below the left costal margin, and the liver was also palpable. There had never been any enlargement of lymphatic glands—or symptoms of glossitis, although her tongue was very red. Once again treatment was stopped, but anaemia returned. Liver extracts of various sources of manufacture were subsequently given intermittently.

In March, 1951, the patient was referred to one of us (J. N. M. C.) at St. George's Hospital, London, and more detailed studies were made. She was then aged 72 and had received an injection of refined liver extract a month before her attendance at hospital.

Clinically she did not appear anaemic—her face being somewhat florid, her conjunctivae injected, and her tongue very red although it was not smooth. While she was of small build there were no signs of wasting and there was no evidence of enlargement of superficial lymphatic glands. The abdomen was protuberant; the spleen was palpable fully a handbreadth below the left costal margin and the liver was also palpably enlarged. There was no evidence of ascites. Tachycardia associated with a collapsing pulse was present; B.P. 185/85 mm. Hg. There was no abnormality of the reflexes. Vibration sense over the shins was poorly appreciated. The urine was normal on routine testing; X-ray examination of the chest, spine, and long bones showed no abnormality apart from senile osteoporosis and osteoarthritis. A radiograph of the chest showed the lung fields to be clear, the transverse diameter of the heart being increased.

MEGALOBlastic ANAEMIA ALTERNATING WITH POLYCYTHAEMIA VERA

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The development of polycythaemia vera in a patient previously proved to have megaloblastic anaemia associated with vitamin-B₁₂ deficiency is a haematological rarity, and only a small number of such cases, varying in degree of detailed study, have been reported. It is of interest to note that Sir William Osler (1903) in his classical paper on polycythaemia mentions a patient who was "as red as a beet" and suspected of having polycythaemia vera, but who had had, in fact, blood-count changes resembling pernicious anaemia. The most recent accounts of the association of pernicious anaemia and polycythaemia vera in the same patient are those of Hinz (1957) and Ellman and