THE EFFECT OF PREVIOUS INJECTIONS OF TUBERCULOPROTEIN ON THE DEVELOPMENT OF TUBERCULIN SENSITIVITY FOLLOWING B.C.G. VACCINATION IN GUINEA-PIGS

S. V. BOYDEN

From the Tuberculosis Immunization Research Centre, c/o Statens Seruminstitut, Copenhagen, Denmark

Received for publication August 9, 1957

INTRODUCTION

THERE is now considerable evidence that both circulating antibodies (Coons, Leduc and Connolly, 1953 ; Ehrich and Harris, 1942 ; Fagraeus, 1948 ; Fagraeus and Grabar, 1953 ; Harris, Harris and Farber, 1954 ; McMaster and Hudack, 1935 ; White, Coons and Connolly, 1955) and the specific host substances or " antibodies " of bacterial allergy (Chase, 1945 ; Metaxas and Metaxas-Buehler, 1954) are formed in lymphoid tissue. It may even be that the same kind of cells are responsible for both types of immune response, but it is not known whether this is so.

In the case of circulating antibodies, it is possible to interfere with the primary response to an antigen by injecting it for the first time together with another antigen which has been previously administered (Barr and Llewellyn-Jones, 1953, 1955). The antibody-forming mechanism is apparently unable to respond properly to the new antigen because it is working almost to full capacity producing the antibodies of the secondary response to the other, previously administered, antigen. If, as we postulate, circulating antibodies are formed by the same cells as are the antibodies of tuberculin type allergy, then it might be possible to demonstrate a similar sort of interference between the production of circulating antibodies and the development of delayed-type skin sensitivity. An experiment designed to test this possibility is described in this paper.

Guinea-pigs were injected with a preparation of unheated tuberculoprotein which is known to cause formation of circulating antibodies (Boyden, 1957*b*) These animals, together with untreated guinea-pigs were then injected with living tubercle bacilli (B.C.G.) and the development of allergy in the two groups was compared. It was found that, when skin tested 11 days after the B.C.G. injection, all the " normal " vaccinated animals gave posititive delayed-type reactions, whereas the animals which had previously been injected with tuberculoprotein gave mild early-type, or Arthus, reactions. At a later skin test delayed-type reactions were seen in both groups. The interpretation of this result will be discussed.

MATERIALS AND METHODS

Guinea-pigs.—Female white guinea-pigs, weighing 300–500 gm., were used. They were bred at Hvidesten, the farm of Statens Seruminstitut.

Tuberculoprotein.—Culture filtrates from the human virulent strain E9656 were Seitz-filtered twice and then concentrated by ultrafiltration and lyophilized (Boyden and Sorkin 1955).

P.P.D. (*RT* 22).—This was obtained from Mr. Magnusson of the Tuberculin Department, Statens Seruminstitut. It had been prepared by trichloracetic acid precipitation of filtrates of heated cultures of tubercle bacilli of the human type (Lind, 1947, 1948).

B.C.G. vaccine was kindly supplied by Dr. Knud Tolderlund of the B.C.G. Department, Statens Seruminstitut.

Bovine albumin.—This was supplied by Dr. A. Hansen of the Physical-Chemical Department, Statens Seruminstitut.

Skin tests.—0·1 ml. of the antigen solution was injected intradermally on the back. The thickness of the skin at the site was measured immediately before the injection (Boyden, 1957*a*). The reaction was read at 3 and 48 hours afterwards, when intensity of erythema was assessed, and the diameter of the reaction and the skin thickness were measured. On each occasion the skin readings were made in random order, and the reader was unaware of the number and group of each animal as he read the test.

Serology.—Sera were tested in the haemolytic modification of the Middlebrook-Dubos test (Middlebrook, 1950; Middlebrook and Dubos, 1948). Details of the technique were as previously described (Boyden, 1957*a*).

EXPERIMENTAL

The effect of Previous Injection of Tuberculoprotein on theDevelopment of tuberculin sensitivity after B.C.G. injection

The experiment was set up with 2 groups of 20 guinea-pigs (Groups 1 and 2) and one group of 10 (Group 3). The treatment of the different groups was as follows:—

1*st day.*—Group 1 received 1 mg. tuberculoprotein in 1 ml. saline subcutaneously.
7*th day.*—Group 1 injected as on 1st day.
10*th day.*—Group 1 injected as on 1st day.
38*th day.*—Groups 1 and 2 were injected with 0·1 ml. of B.C.G. suspension (1 mg. per ml.) intradermally.
48*th day.*—5 ml. blood taken by heart puncture from first 8 animals in each group.
49*th day.*—All animals skin tested with 0·1 mg. P.P.D.
71*st day.*—All animals skin tested with 0·01 mg. P.P.D.

The results of this experiment which are given in Table I, show a big difference between the skin reactions of Group 1 and those of Group 2. Group 1, which had received tuberculoprotein several weeks prior to the B.C.G. injection, reacted with typical early-type reactions, seen particularly in the measurements of thickening of the skin at 3 hr.; by 48 hr. the reaction had almost completely disappeared. In marked contrast, all the animals of Group 2 had good reactions at 48 hr., while at 3 hr. the reactions of this group had been negligible.

In the haemolytic modification of the Middlebrook-Dubos test, all eight sera from Group 1, taken the day before the first skin test, were positive in titres ranging from 1/10 to 1/320 (the antigen fraction used for sensitizing the red cells was unheated and contained both α- and β-haemosensitins, the former being a polysaccharide and the latter probably a protein (Boyden, Sorkin and Engbaek, 1958).

The eight sera from Group 2 were all negative. On the occasion of the second skin test given 33 days after the B.C.G. vaccination, most of the animals in Group 1 showed evidence of an early-type reaction as previously. However, they also had typical tuberculin reactions at 48 hr., which were, on the average, only slightly less intense than those of Group 2.

The possibility that the early-type reaction in Group 1 might have influenced the course of the delayed reaction by causing an immediate increase in capillary

permeability which allowed tuberculoprotein or toxic products of the reaction to escape from the site was tested in the next experiment.

TABLE I.—*Reaction to Skin Tests* 11 *Days after B.C.G. Vaccination and Antibody Titres in Experiment* 1

Number of guinea-pig	Group	Injection	3 hr.		48 hr.		Serum titres
3983	1	Tuberculo-	—	17	±6	10	40
3984		protein and	±18	18	±6	17	40
3985		B.C.G.	±20	19	±5	6	10
3986			±17	29	—	13	40
3987			—	21	±5	14	40
3988			±17	23	—	12	40
3989			±14	18	—	13	20
3990			±20	17	+4	12	320
3991			—	13	++9	14	—
3992			±22	30	±4	11	—
3993			—	10	+5	6	—
3994			±20	19	+7	9	—
3995			±21	29	+5	10	—
3996			+8	17	—	12	—
3997			—	21	++4	4	—
3998			—	8	++11	9	—
3999			—	15	+6	6	—
4000			±21	17	—	11	—
4001			±18	12	++6	13	—
4002			—	10	±6	7	—
Average	..	..	..	18·2	..	10·5	68·8
4003	2	B.C.G.	±15	13	+++13	18	0
4004			±6	9	+++12	18	0
4005			—	11	++++22	15	0
4006			—	10	++++15	22	0
4007			..	..	..	..	0
4008			—	9	+++15	19	0
4009			—	5	++++13	16	0
4010			—	11	+++14	17	0
4011			—	7	+++14	15	—
4012			—	13	+++12	11	—
4013			±5	9	++12	12	—
4014			±7	7	++++16	19	—
4015			—	9	+++16	16	—
4017			—	10	++++15	21	—
4018			±5	9	+++13	15	—
4019			—	10	++++18	20	—
4020			±15	12	++++14	21	—
4021			—	8	++++18	20	—
4022			—	10	++++19	12	—
Average	..	..	..	9·6	..	17·1	0

Key : ± = a trace of erythema.
\+ = pale pink.
++++ = intense erythema.

Note on skin readings.—The colouration of the skin reactions are denoted by signs as explained in the key. In each instance the number which follows the sign indicates the diameter in millimetres of the coloured zone. The number given in the separate column indicates the difference in millimetres between the thickness of the skin at the time of reading and the thickness immediately before the injection of P.P.D. There were no skin reactions in the control (non-vaccinated) group (Group 3).

Serum titres.—The figures refer to the reciprocal of the highest dilution giving complete haemolysis. 0 means there was no complete haemolysis in any tube. — means not tested.

The Effect of an Early-type Reaction on a Delayed-type Reaction at the Same Site

In the second experiment three groups were treated as in previous experiment. A fourth group received injections of bovine albumin instead of tuberculoprotein 4–5 weeks before the B.C.G. injection.

A skin test was performed as previously, 11 days after the injection of B.C.G. On this occasion all animals were skin tested at 3 sites, with (*a*) P.P.D., (*b*) bovine albumin, (*c*) a mixture of P.P.D. and bovine albumin.

1*st day*.—Group 1 injected with 1 mg. tuberculoprotein in 1 ml. saline subcutaneously.
Group 2 injected with 4 mg. bovine albumin in 1 ml. saline subcutaneously.
7*th day*.—As on 1st day.
10*th day*.—As on 1st day.
40*th day*.—Groups 1, 2, and 3 injected with 0·1 ml. B.C.G. suspension (1 mg. per ml.) intradermally.
51*st day*.—All animals skin tested in 3 places with
(*a*) P.P.D. 1 mg. per ml.
(*b*) Bovine albumin 1 mg. per ml.
(*c*) A mixture containing P.P.D. 1 mg. per ml. and bovine albumin 1 mg. per ml.

The results of this test are given in Table II.

In the first place, these results confirm those of the first experiment, in that most of the guinea-pigs injected with tuberculoprotein 4–5 weeks before B.C.G. (Group 1) had negligible reactions at 48 hr., at which time most of the animals which had received only B.C.G. (Group 2) showed strong positive reactions. The animals in the former group had typical early-type reactions to P.P.D.

As was expected, the animals which had been previously injected with bovine albumin (Group 3) also gave typical early-type reactions to bovine albumin, whether it was injected alone or with P.P.D. (These early reactions are more evident in the readings of skin thickness than in the erythema readings.) The significant fact is that in this group the tuberculin reaction (at 48 hr.) was in all cases as strong, or almost as strong, when the tuberculin was injected with bovine albumin as when it was injected alone. Although the Arthus reactions to the bovine albumin were not quite as strong as those against the tuberculin (Group 1), this result strongly suggests that an early-type reaction does not seriously interfere with the development of a tuberculin reaction at the same site.

DISCUSSION

The experiments described above show that guinea-pigs which received injections of tuberculoprotein some weeks prior to B.C.G. injection exhibited no delayed type sensitivity to tuberculin 11 days after vaccination. They did, however, react to P.P.D. with early-type reactions, due to sensitization by the previous injections of tuberculoprotein (Boyden, 1957*a*). In contrast, guinea-pigs which had not received previous injections of tuberculoprotein, gave strong delayed-type reactions to the same dose of P.P.D. 11 days after B.C.G. vaccination.

In trying to interpret this result, it was necessary to consider the possibility that the existence of an early-type reaction due to the sensitization by tuberculoprotein might influence the tuberculin reaction by allowing tuberculoprotein or products of cell damage to escape readily from the site. It has been shown by

TABLE II.—*Skin Reactions in Experiment 2*

Number of guinea-pig	Group	Injection	Reactions to P.P.D.				Reaction to bovine albumin				Reactions to mixture of P.P.D. and bovine albumin			
			3 hr.		48 hr.		3 hr.		48 hr.		3 hr.		48 hr.	
6353	1	Tuberculo-	±10	11	—	3	—	3	—	−1	±12	2	—	2
6354		protein and	±25	22	±7	8	—	5	—	1	±22	22	—	10
6355		B.C.G.	—	9	—	12	—	2	—	0	—	16	—	3
6356			—	13	—	4	—	3	—	0	—	12	—	3
6357			+8 ±26	27	—	3	—	2	—	0	++6 ±30	28	—	5
6358			±14	14	±5	6	—	2	—	2	±15	17	±5	6
6359			±16	16	—	7	—	2	—	0	±20	18	—	9
6360			+6 ±21	24	—	6	—	3	—	−1	+4 ±25	24	—	11
6361			+21	18	—	7	—	2	—	0	+27	21	±5	8
6362			±16	14	±8	5	—	1	—	−1	±16	17	±10	7
Average			..	16·8	..	6·1	..	2·5	..	0	..	17·7	..	6·4
6363	2	Bovine	+3 ±10	6	+++12	11	±12	11	—	−1	—	17	+++15	13
6364		albumin and	—	9	++12	13	±10	15	—	0	±10	16	+12	9
6365		B.C.G.	—	7	+14	9	—	18	—	1	—	16	+11	11
6366			±10	9	+++14	11	±10	16	—	2	±12	18	+++14	17
6367			—	8	+10	7	—	7	—	1	—	13	+10	11
6368			—	8	±3	3	±16	13	—	−1	±16	15	±6	4
6369			—	5	++11	4	—	17	—	−2	—	15	+11	5
6370			—	7	++11	8	—	14	—	0	—	13	+11	6
6371			—	5	++14	6	±13	18	—	0	±16	16	+10	8
6372			—	8	++11	10	±16	14	—	1	+5 ±16	16	++11	14
Average			..	7·2	..	8·2	..	14·3	..	0·1	..	15·5	..	9·8
6373	3	B.C.G.	—	6	++10	10	—	6	—	1	±10	7	+10	7
6374			±14	9	++12	11	—	5	—	1	—	9	++12	8
6375			—	8	+9	10	—	6	—	2	±5	7	+7	6
6476			±11	5	++9	10	—	6	—	2	++7	12	++11	15
6377			—	7	+++12	8	—	4	—	1	±6	11	+++10	10
6378			±6	9	±4	4	—	4	—	−1	—	8	±4	5
6379			—	6	+6	6	—	4	—	−2	±10	11	+9	11
6380			—	7	++11	9	—	4	—	0	+3 ±10	14	++13	16
6381			—	7	++12	16	—	3	—	1	±5	12	++12	16
6382			±6	5	+8	6	±3	4	—	0	±6	11	++9	1
Average			..	6·9	..	9·0	..	4·6	..	0·5	..	10·2	..	9·5
6383	4	No	—	5	—	4	—	5	—	4	±5	8	—	2
6384		treatment	—	6	—	0	—	6	—	−1	±5	8	—	1
6385			±	11	—	2	—	7	—	0	±7	9	—	0
6387			—	5	—	1	—	5	—	0	—	7	—	1
6388			±4	9	—	1	—	8	—	−1	±7	8	—	−1
Average			..	7·2	..	1·6	..	6·2	..	0·4	..	8·0	..	0·6

Key: ± = *a trace of erythema.* + = *pale pink.* ++++ = *intense eythema.*

Note on skin readings.—The colouration of the skin reactions are denoted by signs as explained in the key. In each instance the number which follows the sign indicates the diameter in millimetres of the coloured zone. The number given in the separate column indicates the difference in millimetres between the thickness of the skin at the time of reading and the thickness immediately before the injection of P.P.D.

Pepys (1953) that the intensity of tuberculin reactions can be greatly reduced by injecting histamine diphosphate into the site immediately after the injection of tuberculin. However, the second experiment described above seems to speak against this interpretation; for it was shown that when bovine albumin and P.P.D. were injected into the same site in animals sensitized to the former antigen, the early-type reactions to bovine albumin had little, if any, influence on the intensity of the subsequent delayed reaction to P.P.D.

There seem to be two other possible interpretations of the results, and it is difficult at the present state of our knowledge to choose between them. The first, which was in mind when the experiment was set up, is that the injection of B.C.G. into the animals which had previously received tuberculoprotein causes a secondary response of circulating antibodies against tuberculoproteins, thereby interfering with the ability of the antibody-producing mechanism to form " allergy antibodies " against these antigens.

The other possibility is that, although the " allergy antibody " is formed at the same rate in both groups of guinea-pigs, there is competition between circulating antibodies and " allergy antibodies " for antigen in the animals which had previously received tuberculoprotein. When the level of the allergy is relatively low, as it is 11 days after vaccination, circulating antibodies in high titre might well tend to depress the delayed allergic reaction by combining with and " neutralizing " the antigen.

The first interpretation is in accord with the hypothesis that circulating gamma globulin antibodies, and the " antibodies " of allergy are produced by the same mechanism and in the same cells. Possibly the factor which determines which kind of antibody will be formed is the particular form in which the antigen is received by the antibody-forming cells. If protein antigen is injected in solution, it is likely to reach these cells in an unmodified form. But in tuberculous infection, for example, the protein antigen must necessarily pass through macrophages before reaching the antibody-forming cells and may well be modified in some way by the process. The cells, on receiving this modified antigen, could be expected to respond to it differently (Boyden, 1957*b*).

The second interpretation, that the antibodies responsible for the Arthus type of reaction compete for the antigen with those responsible for tuberculin allergy, raises the question as to what extent such competition might play a part in tuberculous disease. Is it possible that in tuberculosis high titres of circulating antibodies which are thought to be ineffective against tubercle bacilli, might actually be prejudicial to the interests of the host by " protecting " the bacilli against the possibly more anti-bacillary effects of the allergy antibodies of the same specificity?

The various problems discussed above are being investigated in a further series of experiments.

SUMMARY

Guinea-pigs skin tested with tuberculin (P.P.D.) 11 days after B.C.G. vaccination gave strong delayed-type skin reactions. However, guinea-pigs which had received injections of tuberculoprotein 4–5 weeks before the injection of B.C.G. were not tuberculin positive 11 days after vaccination. They did, however, show mild skin reactions of the early type due to sensitization by tuberculoprotein.

An early-type skin reaction to bovine albumin at the same site as a tuberculin reaction had little or no influence on the latter reaction.

The interpretation of these results is discussed.

This work was carried out with the technical assistance of Miss Gabrielle Hurter and Miss Jytte Mensen.

REFERENCES

BARR, M. AND LLEWELLYN-JONES, M.—(1953) *Brit. J. exp. Path.*, **34**, 12 and 233.—(1955) *Ibid.*, **36**, 147.
BOYDEN, S. V.—(1957*a*) *Int. Arch. Allergy*, **10**, 65.
Idem.—(1957*b*) ' Progress in Allergy ', in press.
Idem AND SORKIN, E.—(1955) *J. Immunol.*, **75**, 15.
BOYDEN, S. V., SORKIN, E., AND ENGBACK, H. C.—(1958) *Acta path. micriobiol. scand.*, in press.
CHASE, M. W.—(1945) *Proc. Soc. exp. Biol., N.Y.*, **59**, 134.
COONS, A. H., LEDUC, E. H. AND CONNOLLY, J. M.—(1953) *Fed. Proc.*, **12**, 439.
EHRICH, W. E. AND HARRIS, T. N.—(1942) *J. exp. Med.*, **76**, 335.
FAGRAEUS, A.—(1948) *Acta med. scand.*, **130**, suppl. 204.
Idem AND GRABAR, P.—(1953) *Ann. Inst. Pasteur*, **85**, 34.
HARRIS, S., HARRIS, T. N. AND FARBER, M. B.—(1954) *J. Immunol.*, **72**, 148.
LIND, P.—(1947) *Acta tuberc. scand.*, **21**, 111.—(1948) *Ibid.*, **22**, 287.
MCMASTER, P. D. AND HUDACK, S. S.—(1935) *J. exp. Med.*, **61**, 783.
METAXAS, M. N. AND METAXAS-BUEHLER, M.—(1954) *Schweiz. Z. Path.*, **17**, 128.
MIDDLEBROOK, G.—(1950) *J. clin. Invest.*, **29**, 1480.
Idem AND DUBOS, R. J.—(1948) *J. exp. Med.*, **88**, 521.
PEPYS, J.—(1953) *Acta allerg., Kbh.*, **6**, 265.
WHITE, R. G., COONS, A. H. AND CONNOLLY, J. M.—(1955) *J. exp. Med.*, **102**, 73.