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REFERENCES

- Barnett, A. J. (1956). *Aust. Ann. Med.*, 5, 274.
 Dustan, H. P., Schneekloth, R. E., Corcoran, A. C., and Page, I. H. (1958). *Circulation*, 18, 644.
 Harington, M., Kincaid-Smith, P., and McMichael, J. (1959). *Brit. med. J.*, 2, 969.
 Hill, A. B. (1955). *Principles of Medical Statistics*, 6th ed. Lancet, London.
 Keith, N. M., Wagener, H. P., and Barker, N. W. (1939). *Amer. J. med. Sci.*, 197, 332.
 Moyer, J. H. (1959). *Hypertension* (The First Hahnemann Symposium on Hypertensive Disease). Saunders, Philadelphia and London.
 Newman, M. J. D., and Robertson, J. I. S. (1959). *Brit. med. J.*, 1, 1368.
 Perry, H. M., jun., and Schroeder, H. A. (1958). *Arch. intern. Med.*, 102, 418.
 Smirk, F. H. (1954). *Brit. med. J.*, 1, 717.
 — (1957). *High Arterial Pressure*. Blackwell, Oxford.
 — Hamilton, M., Doyle, A. E., and McQueen, E. G. (1958). *Amer. J. Cardiol.*, 1, 143.

COMPARATIVE TUBERCULIN-TESTING OF HUMAN SUBJECTS

A METHOD OF DIFFERENTIATING THE TYPE OF VIRULENT MYCOBACTERIAL INFECTION

BY

C. J. STEWART, M.D.

D. P. F. EMBLETON, M.R.C.S., L.R.C.P.

AND

D. F. VAN ZWANENBERG, D.M., D.P.H., D.C.H.

From the East Suffolk and Ipswich Chest Service

The successful discovery of the source of tuberculous infection by single or repeated tuberculin-testing of adults (Gedde-Dahl, 1952) and schoolchildren (Tattersall, 1952; MacDougall *et al.*, 1953; Davies, 1955) has been reported. Persistent attempts over the last six years to repeat this technique in Ipswich and East Suffolk resulted in the tracing of the source of infection in about a fifth of the children tuberculin-positive at their first test, and in a third of those converting their tuberculin test (van Zwanenberg, 1960). It was suspected that much of the infection unaccounted for might be due to milk infection with the bovine type of *Mycobacterium tuberculosis*. In such circumstances a simple method of comparative tuberculin-testing as is used by veterinary surgeons to determine the type of mycobacterial infection in cattle would be of great value. Testing simultaneously with avian and mammalian tuberculin they are able to differentiate virulent mycobacterial infection from tuberculin hypersensitivity due to other causes (Francis, 1958). In his report on the preparation of Weybridge P.P.D., Green (1946) showed that guinea-pigs hypersensitized to different strains of *Mycobacterium* gave the largest reactions to the homologous P.P.D. tuberculin.

Until recently there have been very few reports on comparative tuberculin-testing of human subjects. Avian and human tuberculin have been used by Edwards *et al.* (1953) and by Kuper (1958) in an attempt to differentiate between hypersensitivity due to virulent *Mycobacterium tuberculosis* and "non-tuberculous" hypersensitivity. The work of Enell (1955) and Lorber (1957)

has shown that reactions to B.C.G. tuberculin are larger than those to human P.P.D. in B.C.G.-vaccinated persons. More recently Edwards and Palmer (1958) have shown that patients harbouring anonymous (Battey) *Mycobacterium tuberculosis* give larger reactions to P.P.D. prepared from that organism than to P.P.D.-S. (human).

Without knowledge of this recent work, we carried out some preliminary tuberculin-testing of adults and children with bovine and human P.P.D. in 1957. The result suggested that comparative testing might be of value in indicating the type of infecting *Mycobacterium*. This paper describes the results of the subsequent tuberculin-testing of schoolchildren in East Suffolk County and Norwich with human and bovine P.P.D. and relates the findings to the known facts concerning tuberculous disease and infection in each district.

Population Studied

The subjects in this survey were schoolchildren aged 5 to 18 living in six districts in East Suffolk County and one group from the City of Norwich. The schoolchildren from 5 to 11 years old live in or near the town or village in which the school is situated. Older children come from a wider area surrounding the school. In six districts over 80% of the school population accepted and completed the tests. In Hartismere only 78% of the total school population completed the tests; 12% attend schools outside its boundaries and have not as yet been tested.

East Suffolk is predominantly rural. The industry and occupations are mainly concerned with farming. Of the seven districts covered by this investigation, only Hartismere (16,940 pop.) is entirely rural and has no occupation other than farming, most of which is arable, although in the north along the Waveney Valley there are a number of dairy farms. Four of the districts—Woodbridge (5,620 pop.), Bungay (3,610 pop.), Beccles (7,000 pop.), and Stowmarket (7,580 pop.)—are small market towns surrounded by arable and mixed farming areas. In Beccles there is a printing works employing 1,000, Stowmarket has a paint factory employing 1,000 and an iron foundry employing 700. Felixstowe is a residential coastal resort with a large number of summer visitors and no factories. Norwich City (118,000 pop.) is the county town of Norfolk. It is a large trading centre with mixed light and heavy industry.

Method and Materials

All children received two Heaf multiple-puncture tuberculin tests. Bovine P.P.D. 1 mg./ml. (Weybridge) was applied to the right forearm, and human P.P.D. 2 mg./ml. (Weybridge) to the left forearm. This human P.P.D. is the standard preparation issued by the Weybridge laboratory of the Ministry of Agriculture, Fisheries and Food for the routine tuberculin-testing of cattle and for the commercial dispensing and dilution, and is issued by the Ministry of Health to local authorities for the tuberculin-testing of schoolchildren. It is prepared from three strains of human-type *Mycobacterium* designated C, D.T., and P.N. that have been maintained separately in the laboratory for a number of years (Green, 1946). The bovine P.P.D. is prepared in the same laboratory from a single strain "OTTO" (A. B. Paterson, 1960, personal communication). It is issued in 1 mg./ml. strength for research and experimental work only. The relative strength of the tuberculins is that arrived at by Green (1946) as a

TABLE I.—Number and Percentage of Bovine and Human P.P.D. Reactions and Their Mean Size

Group of School-children Tested	No. Completing Test	Giving No Reaction to Both Tuberculins		Reacting to Both Tuberculins		Reacting to Bovine P.P.D. Only		Reacting to Human P.P.D. Only		Mean Size of Reaction (mm.)	
		No.	%	No.	%	No.	%	No.	%	Bovine P.P.D.	Human P.P.D.
Stowmarket ..	1,271	803	63.2	264	20.8	203	15.9	1	0.1	9.2	8.4
Bungay ..	315	251	79.6	43	13.6	21	6.7	0	0	9.0	6.3
Hartismere ..	1,776	1,492	84.0	222	12.5	57	3.2	5	0.3	8.4	7.4
Woodbridge ..	954	810	83.9	102	10.6	41	4.3	1	0.1	6.8	7.8
Beccles ..	1,758	1,492	84.9	206	11.5	40	3.0	20	1.5	7.4	7.9
Felixstowe ..	1,842	1,490	80.9	258	14.0	29	1.6	65	3.5	7.8	9.9
Norwich ..	923	863	93.5	30	3.3	6	0.7	24	2.6	4.0	4.7
Total ..	8,839	7,201		1,125		397		116			

result of tuberculin-testing guinea-pigs infected with specific strains of *Mycobacterium* and tested with P.P.D.s derived from these strains. Defining the "specificity factor" as the number of weight units of heterologous P.P.D. required to elicit the same intensity of skin reaction as one weight-unit of homologous P.P.D., he found that in guinea-pigs hypersensitized with the human strain of *Mycobacterium* it required twice as much human P.P.D. as bovine P.P.D. to elicit the same size of reaction.

Two of the new type of Heaf multiple-puncture machines (Carpenter and Stewart, 1959), which were kept separate for each tuberculin were used throughout. The depth of puncture used was 1 mm. In all districts except Norwich the tests were performed and the reaction was read a week later by a nurse who had had considerable previous experience. In Norwich the testing was done by Dr. P. Sutton. The size of the reaction was measured with a transparent plastic gauge* imprinted with circles, the diameter of which increased by 1 mm. up to 10 mm. and then at 2 mm. and 5 mm. intervals up to 30 mm. (Stewart and Carpenter, 1961). For papule and ring reactions the external and internal diameters were measured and the width of the ring or circle of papules was recorded.

Tuberculin-testing with Human and Bovine P.P.D.

Between May, 1958, and April, 1959, over 8,000 schoolchildren have been tested simultaneously with bovine and human P.P.D. The results of testing 7,916 in six districts of East Suffolk and 923 in Norwich City are presented in Table I. In three of the districts—Hartismere, Woodbridge, and Beccles—they are similar. Approximately 84% of children failed to react to either tuberculin; between 10.5% and 12.5% reacted to both; 3 to 4.5% reacted to bovine P.P.D. alone, and between 0.3 and 1.5% reacted to human P.P.D. alone. In Stowmarket there were many more reactors to one or both tuberculins (36.8%); 20.8% reacted to both tuberculins, 15.9% reacted to bovine P.P.D. alone, and 0.1% to human P.P.D. alone. In Bungay a total of 20.3% reacted—13.6% to both tuberculins, 6.7% to bovine P.P.D. alone, and none to human P.P.D. alone. In Felixstowe 19.1% reacted to one or both tuberculins, about the same percentage as in Bungay; this is much less than in Stowmarket but slightly more than in the other three districts. However, the proportion reacting to human tuberculin alone (3.5%) was more than twice that of any other district except Norwich. The proportion of reactors to bovine tuberculin alone (1.6%) was the lowest of the six districts in Suffolk. In Norwich only 6.6% of the children reacted to one or both tuberculins, but the proportion reacting to human P.P.D. alone—2.6%—was relatively the highest in any

area. The mean size of bovine P.P.D. reactions was largest in Stowmarket (9.2 mm.), followed closely by Bungay (9.0 mm.), and that for human P.P.D. reactions was in Felixstowe (9.9 mm.) (Table I).

Tables II and III compare the distribution of reactions to bovine and human tuberculin according to size in Stowmarket and Felixstowe. In both areas it will be

TABLE II.—Correlation Table Giving the Number and Percentage of Reactions to Bovine and Human P.P.D. by Size. Stowmarket School Children All Ages

Bovine Reaction Size (mm.)	Human Reaction Size (mm.)										Total	
	0	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15+	No.	%	
0	574	1	—	—	—	—	—	—	—	575	56.3	
1-2	4	1	—	—	—	—	—	—	—	5	0.4	
3-4	11	—	—	—	—	—	—	—	—	11	1.1	
5-6	84	—	5	4	—	—	—	—	—	93	9.1	
7-8	101	—	10	13	25	—	—	—	—	149	14.6	
9-10	2	—	—	1	14	4	—	—	—	21	2.1	
11-12	—	—	—	1	30	18	19	—	—	68	6.6	
13-14	—	—	—	1	13	8	57	7	—	86	8.4	
15+	—	—	—	—	—	—	6	—	6	12	1.1	
Total %	77.6	0.1	1.4	1.9	8.0	0.6	2.9	8.0	0.5	1,020		

Both bovine and human reactions, of 1-6 mm. presented as papules, of 6-8 mm. as rings, and of 8-15+ mm. as plaques.

TABLE III.—Correlation Table Giving the Number and Percentage of Reactions to Bovine and Human P.P.D. by Size. Felixstowe School Children All Ages

Bovine Reaction Size (mm.)	Human Reaction Size (mm.)										Total	
	0	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15+	No.	%	
0	1,570	2	15	23	23	1	1	1	—	1,636	83.1	
1-2	1	—	—	1	—	—	—	—	—	2	0.1	
3-4	13	1	3	6	5	2	1	—	—	31	1.5	
5-6	13	—	3	56	34	4	8	5	2	125	6.3	
7-8	13	—	—	10	64	7	27	6	1	128	6.5	
9-10	—	—	—	—	7	3	9	4	2	25	1.2	
11-12	—	—	—	—	1	1	5	6	4	17	0.8	
13-14	—	—	—	—	—	—	2	1	—	3	0.1	
15+	—	—	—	—	—	—	1	—	—	1	0.1	
Total %	1,610 81.8	3 0.1	21 1.0	96 4.8	134 6.8	18 0.9	54 2.7	23 1.1	9 0.4	1,968		

Presentation of reactions as in Table II.

seen that there is a considerable degree of cross-reaction to the two tuberculins. The Stowmarket children reacting to both tuberculins have larger reactions to bovine P.P.D. than to human P.P.D. In Felixstowe, however, more children gave larger reactions to human P.P.D.

To summarize, Stowmarket and Bungay have more reactors than any other district; almost all of these react to bovine P.P.D. and give larger reactions to bovine than to human P.P.D. Felixstowe has slightly more reactors than the remaining districts, but a higher proportion react to human P.P.D. alone, and human P.P.D. reactions tend to be larger than those to bovine P.P.D. Norwich has the lowest incidence of reactors but proportionately the highest incidence of reactors to human P.P.D. The districts of Hartismere, Beccles, and

*Obtainable from Messrs. C. L. Bencard Ltd., Minerva Road, London N.W.10.

Woodbridge hold a position intermediate between the two extremes.

Known History of Infected Milk Supplies

There was an outbreak of tuberculous disease and infection originating from one dairy in Stowmarket in 1953-4. This dairy supplied about 12% of the milk consumed in the town. It is probable that another dairy was supplying infected milk in 1950 (van Zwanenberg *et al.*, 1956). In Bungay three dairies supplied milk up to March, 1953. In 1952 bovine-type tubercle bacilli were isolated on two occasions from the milk supplied by one of these dairies. This dairy supplied the town with 80 gallons (364 litres), or about 10% of the daily milk consumption. This dairy dispersed its herd in 1955. So far as is known, no infected milk has been supplied on the same scale in the other areas for the past fifteen years. The Felixstowe milk supply is known to have been "safe" since 1939, as has that of Norwich.

In 1954 milk infection in Stowmarket resulted in 21 cases of tuberculous disease: 17 of cervical adenitis, 2 of tuberculous peritonitis, 1 tuberculous elbow, and 1 tuberculous hip. Seventeen of these children have had pathological proof of the diagnosis after operation, and in six acid-fast bacilli have been seen in the pus from the lesions. Thirteen of these patients (cervical glands 11, and peritonitis 2) have recently had simultaneous Heaf tests using human P.P.D. 0.2 mg./ml. and bovine P.P.D. 0.1 mg./ml. These dilute tuberculins were used in order to avoid severe reactions. In one patient there was no difference in the two tests. In a further two the measurement of the reaction was the same for both tests, although in the bovine test it appeared to be the more prominent. In the other 10 patients the bovine reaction was definitely larger, and in four of them was accompanied by ulceration, which was not present with the human test. Several other children and young adults in Stowmarket now show x-ray evidence of calcified mesenteric glands. In the whole of East Suffolk County covered by the Ipswich Chest Clinic the number of new cases of cervical adenitis and non-pulmonary tuberculosis arising since 1954 has been relatively few (Table IV).

Incidence of Pulmonary and Non-pulmonary Tuberculosis

The incidence of registered tuberculosis in 1953 in Bungay, Stowmarket, Felixstowe, the rest of East Suffolk covered by Ipswich Chest Clinic (92,000 pop.), and the City of Norwich is shown in Table IV.

TABLE IV.—Incidence of Registered Cases of Pulmonary and Non-pulmonary Tuberculosis in 1953 and the New Cases Registered Between 1954 and 1959 Inclusive

District	Incidence of Registered Tuberculosis in 1953 per 1,000 Population			Incidence of New Cases of Tuberculosis Registered during 1954-9 per 1,000 Population		
	Pulmonary	Non-pulmonary	Total	Pulmonary	Non-pulmonary	Total
East Suffolk*	3.2	0.8	4.0	1.8	0.7	2.5
Bungay ..	5.4	2.6	8.0	3.6	0.8	4.4
Stowmarket ..	4.7	3.3	8.0	3.7	2.5	6.2
Felixstowe ..	3.8	1.1	4.9	2.9	0.3	3.2
Norwich ..	6.2	1.1	7.3	3.1	0.5	3.6

* Excluding Felixstowe, Stowmarket, and Bungay.

Bungay and Stowmarket had more tuberculosis than the other districts. Most of this high incidence is due to an excess of non-pulmonary tuberculosis. Bungay also had a comparatively high incidence of pulmonary tuberculosis. Norwich had the highest incidence of

pulmonary tuberculosis and shared with Felixstowe a low incidence of non-pulmonary tuberculosis. The amount of fresh disease occurring during 1954-9 was highest in Bungay and Stowmarket. Again, this is largely accounted for by the excess of non-pulmonary disease in these two districts.

There was a small difference in the amount of fresh pulmonary tuberculosis in all the districts, ranging from 2.9 per 1,000 in Felixstowe to 3.7 per 1,000 in Stowmarket. Only in East Suffolk was the incidence low—1.8 per 1,000. These figures show that in districts with a high incidence of non-pulmonary tuberculosis there are more schoolchildren giving reaction to bovine P.P.D. than to human P.P.D. whereas in districts with a low incidence of non-pulmonary tuberculosis fewer children react to bovine than to human P.P.D. It will also be noted (Table I) that the mean size of the reaction to bovine and human P.P.D. follows the relative incidence of pulmonary and non-pulmonary tuberculosis. In Bungay and Stowmarket the mean size of bovine reaction is greatest, and in Norwich and Felixstowe the mean size of human reaction is greatest.

Age Distribution of Reaction to Human and Bovine P.P.D.s

The age distribution of the reactors to human and bovine P.P.D. in Stowmarket in 1958 and in Felixstowe in 1959 is shown in Figs. 1 and 2. At all ages Felixstowe has a lower incidence of reactors to both tuberculins. The incidence of reactions to the two tuberculins is not very different up to the age of 11; after that the curves diverge slightly, the incidence of human reactors exceeding that of the bovine reactors. This is in complete contrast to the curves for Stowmarket in 1958, where at all ages the incidence of bovine

FIG. 1.—Percentage incidence by age of positive Heaf M.P. reactions to human P.P.D. in 1955, 1956, and 1958, and to bovine P.P.D. in 1958 in schoolchildren in Stowmarket.

*The discrepancy between these totals and those recorded in Tables I, II, and III is due to: (1) the figures for those aged 16 and over are not recorded on the graphs; and (2) the figures in the graphs apply to children living in the urban district and exclude those in surrounding areas who come into the town to go to school.

reactors exceeds that of human reactors, and from the age of 9 onwards there is a steep and rising incidence of bovine reactors. This diverging curve showing a high incidence of bovine reactors is seen to a less extent in Bungay schoolchildren from the age of 6, when the incidence for both tuberculin is 2% and rises to 20% for human reactors and 39% for bovine reactors at age of 9. The Norwich curves are very similar to those of Felixstowe in that human reactors exceed bovine reactors at all ages, but the incidence of reactors to both tuberculin is the lowest of any of the areas. Felixstowe and Norwich, two urban populations with a clean milk

FIG. 2.—Percentage incidence by age of positive Heaf M.P. reactions to human P.P.D. in 1957 and 1959 and bovine P.P.D. in 1959 in schoolchildren in Felixstowe. *Footnote as in Fig. 1.

supply, both have a low incidence of infection and a low incidence of non-pulmonary tuberculosis. In contrast, Stowmarket and Bungay, both towns with a known infected milk supply in the last few years, have a high incidence of infection, the bovine P.P.D. reactors at all ages exceeding those reacting to human P.P.D., and both districts have a relatively high incidence of non-pulmonary tuberculous disease.

Incidence of Infection as Shown by Previous Tuberculin-testing

The Felixstowe and Stowmarket school populations have previously been tested with human P.P.D. given by the Heaf technique. Stowmarket children were tested in 1955, 1956, and 1958. From 1955 to 1958 34 children became tuberculin-positive and 10 have reverted to negative. The curve of the age incidence of reactors (Fig. 1) at each testing is similar, as would be expected where there has been a negligible amount of fresh infection. Only 0.2% of the population was infected from 1955 to 1958. The Felixstowe population was tested in November, 1957, and again in March, 1959 (Fig. 2). In this 18-months period 62 (0.34%) of the school population became positive; 40 of these children attended one school. In nearly all cases these converters gave larger reactions to human P.P.D. or reacted to it alone. The source of a few cases is known, but the main source of this infection has not yet been identified. This infection in Felixstowe has had the effect of displacing the curve showing the age incidence of human reactors to the left, indicating a higher incidence at all ages: the increased incidence being greatest over the age of 11, owing to the infection occurring mainly in one secondary school.

Incidence of Childhood Infection Related to Infected Milk Supplies

The Stowmarket schoolchildren over the age of 9 were exposed to the 1950 and 1953-4 milk-borne infections and those children over 6 years old were exposed to the 1953-4 infection, since when there is evidence of only a negligible amount of fresh infection in the population. We believe it is these milk-borne infections of 1950 and 1953-4 that are responsible for the high incidence of infection, the higher incidence of bovine P.P.D. reactors than human P.P.D. reactors, and the high incidence of non-pulmonary tuberculosis. In the Felixstowe children, on the other hand, the incidence of tuberculous infection is lower, the incidence of reactors to human P.P.D. exceeds that of bovine P.P.D., human P.P.D. reactions tend to be larger than bovine reactions, and the incidence of non-pulmonary tuberculosis is low. The milk supply is "clean," but there is evidence of an outbreak of tuberculous infection believed to be of human origin in one school between November, 1957, and March, 1959.

Conclusions

The results presented in this paper show that when school populations are simultaneously tested with human and bovine P.P.D., using the Heaf technique, there are varying patterns of reactions to the two tuberculin in different districts. These patterns, which are dependent upon the size of the reactions and the incidence by age of reactors to the two tuberculin, demonstrate that there is a definite qualitative difference in the two P.P.D.s which can be related to the known history of milk-borne infection in the district and the incidence of pulmonary and non-pulmonary tuberculous disease. This suggests that comparative tuberculin-testing with these two tuberculin can in certain conditions indicate the type of mycobacterial infection.

Discussion

Although the evidence submitted in support of the conclusions of this investigation appears satisfactory there are limitations in our knowledge of the methods and the materials used which must occur with any new line of approach to a problem, particularly when much of this evidence is gained in retrospect.

In all but one district (Norwich) one nurse was responsible for the Heaf testing and reading. It could, therefore, be claimed that the results are biased. However, at the time neither the nurse nor we were fully aware of the state of the milk supply in Felixstowe or Bungay or of the relative incidence of pulmonary and non-pulmonary tuberculous disease in any of the districts tested.

Limitation of Method and Materials

A large part of the evidence in support of the conclusions rests on the assumption that most non-pulmonary tuberculous disease is due to the bovine type *Myc. tuberculosis* (Wilson *et al.*, 1952). Unfortunately the organisms from the Stowmarket children found to be suffering from non-pulmonary tuberculosis were not cultured or typed. So far as is known, all mycobacteria isolated from patients suffering from pulmonary tuberculosis in East Suffolk in the last 12 years have been of human type. It would therefore be expected, using the technique of comparative tuberculin-testing

described in this paper, that most patients with pulmonary tuberculosis would give more and larger reactions to human than to bovine P.P.D. Limited experience has not shown any clear differentiation. The reaction to both tuberculin have been large and have shown only a small difference in size at the conventional time of reading (Table V). There are a number of

TABLE V.—Mean Size of Heaf Reaction to Human and Bovine P.P.D. Read at Different Times After Testing

Time of Reading Reactions	No. Read	Mean Size of Reaction in mm.	
		Human	Bovine
24 hours	13	13.7	11.4
72 "	13	13.5	13.0
7 days	7	9.1	9.7

factors that may be responsible for this. Large Heaf reactions are mainly concentrated in the size 8–16 mm. diameter (Fig. 3). Mantoux reactions in the same subjects are distributed over a wider range, 8–28 mm. This narrow grouping of large Heaf reactions is inherent

FIG. 3.—Reaction size in mm. to a 10 T.U. Mantoux test and a Heaf M.P. 2 mg./ml. test given simultaneously to 50 mental hospital patients.

in the test and is probably due to the overlapping of induration from the six points of puncture, whereas in the Mantoux test the induration spreads concentrically from a single point of injection. This concentrated grouping of the Heaf reaction makes it more difficult to measure differences which may be apparent when inspecting the reaction. The apparent successful differentiation in schoolchildren is probably due to their relatively low level of hypersensitivity, giving Heaf reactions whose sizes are largely in the lower end of the scale.

Varying Intensity of Reaction with Age

With increasing age a greater number of subjects react to both tuberculin; their reactions to both bovine and human P.P.D. become larger and the differentiation less clear. The increasing incidence of reactions with age is due to the greater chance of infection. We have found in one village that the incidence of human reaction increases from 8% at the age of 15 to 70% at age 30, and bovine reactions from 32% at 15 to 73% at 30. The increased intensity of

TABLE VI.—Mean Size of Hypersensitivity Reaction to Human and Bovine P.P.D. in Different Age Groups

Group	No. of Reactors	Mean Size of Reactions (mm.)	
		Human P.P.D.	Bovine P.P.D.
Primary-school children	260	4.6	6.7
Secondary-school " "	850	6.7	8.6
Adults	442	9.2	12.0

reaction with age (Table VI) to both tuberculin could be due to the stimulating effect of multiple infections with the same or different mycobacteria or to the host's altered antibody response with increasing age. Whichever factor is operating it is clear that the virgin soil of childhood is the most suitable for the epidemiological study of comparative tuberculin-testing.

Weybridge Tuberculin

A great deal of work has been reported on the comparative tuberculin-testing of cattle with mammalian and avian P.P.D. in order to differentiate tuberculous from non-tuberculous disease (Cobbett and Griffith, 1913; Francis, 1958; Edwards and Edwards, 1960). Comparative tuberculin-testing with human and bovine tuberculin in man (Fenger *et al.*, 1934; McCarter *et al.*, 1938) and cattle (Paterson, 1948) has received very little attention, and the results in human subjects have been inconclusive, owing probably to a number of factors, particularly the quality of the tuberculin used in the past (Green, 1946). There was therefore very little information available at the start of this investigation. The human Weybridge tuberculin used in this survey has been described in detail by Green (1946). The human-type *Myco. tuberculosis* used in the production of this P.P.D. gives a protein yield five times greater than that of the bovine type. It is for this reason that bovine P.P.D. has not been used for comparative testing of cattle.

The bovine-type *Myco. OTTO*, originally obtained from Copenhagen and used in the preparation of the P.P.D. for this investigation, has been maintained in the Weybridge laboratory for some years. The P.P.D. prepared from it has been used in tuberculin investigations and has been issued at the strength of 1 mg./ml. for research purposes. There are no published British reports on this P.P.D. (Paterson, 1960, personal communication). Svenkerud (1955) investigated the skin hypersensitivity induced by, and the antigenic properties of tuberculin prepared from, different strains of human, bovine, and avian type *Myco. tuberculosis* kept in different laboratories throughout the world for the production of tuberculin. He was able to show in calves and guinea-pigs that tuberculin prepared from OTTO was antigenically closely related to most human strains. Of the bovine strains examined it was one of the best producers of tuberculin and antigenically the most potent. Reporting on another bovine P.P.D. prepared from laboratory strains A.N.5 and C.45 at Weybridge, Paterson (1948) mentioned that in cattle it gave more "specific" reactions for virulent infection than does mammalian P.P.D.

It has already been mentioned that Green (1946) showed that bovine P.P.D. gives larger reactions in human hypersensitized guinea-pigs than does human P.P.D. This has been allowed for in this investigation in that the strength of human P.P.D., 2 mg./ml., was twice that of the bovine P.P.D., 1 mg./ml. However, there is no evidence that this is the correct ratio of strengths for the human subject. It may be that

antibody response of the human subject to the two tuberculins is both qualitatively and quantitatively different from that of the guinea-pig.

Heaf Tuberculin Test

As well as the use of improved and possibly more specific P.P.D.s, it is possible that the Heaf method of testing, using a much more concentrated tuberculin than the Mantoux test, is better able to elicit specific reactions. It is known that dilute P.P.D.s lose a good deal of their potency because of adsorption of the antigen on to the glass container (B.T.A., 1957). There is some evidence that in dilute solution the antigenically specific fractions of the P.P.D. are more easily adsorbed than the antigens common to other mycobacteria (Waalder *et al.*, 1958). In this way the specificity of the P.P.D. is reduced or lost in dilute solutions such as those used in the Mantoux test.

At the beginning of the investigation little was known of the Heaf test as a quantitative test (B.T.A., 1959). This test was used because it is readily accepted by schoolchildren and their parents, and, once having been used, it is difficult to return to the Mantoux test. Its other advantages are the ease and speed of administration. However, its use would appear to have been justified by the results. The advantage of the greater spread of reaction size to the Mantoux test has been mentioned earlier. There is evidence that in other respects the Heaf test is comparable and may have technical advantages over the Mantoux test (Stewart *et al.*, 1958; B.T.A., 1959; Carpenter and Stewart, 1959).

The reading of the Heaf test on the seventh day is a well-established practice (Stewart *et al.*, 1958; B.T.A., 1959) and this has earned for the test the name "market-day test." It is, however, not certain that this is the optimum time when examining for specific hypersensitivity as in comparative testing. Several authors (Lind and Holme, 1943; Hagberg, 1950) have reported that children vaccinated with B.C.G., and subsequently tested with B.C.G. tuberculin, gave reactions which appeared earlier and were more pronounced than reactions to P.P.D. and O.T. Tarshis and Frisch (1952), investigating the interrelationship between skin hypersensitivity to different tuberculins prepared from virulent and non-pathogenic mycobacteria, showed that guinea-pigs hypersensitized to these organisms gave "typical" reactions to the specific tuberculin but only weak transient reactions to other P.P.D.s.

Further evidence that reactions to the specific tuberculin or antigen may appear earlier is given by the results of testing 30 subjects, who had no naturally acquired hypersensitivity in that they gave no reaction to a 10 T.U. Mantoux test, a standard P.P.D. Heaf test, and B.C.G. vaccine 20 mg./ml. given by the Heaf technique. Six months later they were all again tested with the same three antigens given by the same technique. On this occasion (Table VII) 16 gave prompt

reactions to the B.C.G. test at 72 hours whereas only five reacted to the P.P.D. Heaf test and four to the Mantoux. On the seventh day 17 reacted to the B.C.G. test, nine to the Heaf test, and six to the Mantoux test. The hypersensitivity in these subjects had presumably been induced by the first B.C.G. test.

The subsequent testing showed that this hypersensitivity was revealed promptly and more completely by the B.C.G. antigen than by O.T. or P.P.D. (Weybridge). Also it will be seen (Table V) that the patients suffering from pulmonary tuberculosis and tested with human and bovine P.P.D. gave larger reactions to human P.P.D. than to bovine P.P.D. at 24 hours, at 72 hours there was very little difference in the size, and at seven days both reactions were waning, those to human P.P.D. more rapidly than those to bovine P.P.D. There is therefore a need for further investigation to determine the optimum time to read the reactions when studying specific hypersensitivity.

Mycobacterial Hypersensitivity Other than that Due to Virulent Organisms

The results of this investigation have demonstrated that some subjects possess tuberculin hypersensitivity more easily elicited by bovine P.P.D.; in fact, much of it is not revealed by normal doses of human P.P.D. given by the Heaf test. Pollock *et al.* (1959) have shown that "high dose" hypersensitivity in this country is probably the result of naturally acquired tuberculous infection. Edwards *et al.* (1948) have, however, suggested that in certain parts of the U.S.A. (Edwards *et al.*, 1959) and probably elsewhere (Palmer and Edwards, 1955) some of this "high dose" hypersensitivity is due to infection with mycobacteria other than the usual virulent human and bovine strains. The excess of bovine reactors revealed by this investigation almost certainly belongs to the so-called "high dose" hypersensitivity group; however, it appears to be related to the history of milk infection in the districts and to the known incidence of non-pulmonary tuberculosis. It would therefore be unnecessary to suggest a non-virulent or non-pathogenic mycobacterial infection as an alternative explanation.

In spite of the deficiencies in the design of this investigation and our lack of knowledge of the technique and materials of comparative tuberculin-testing there is evidence in the childhood population of East Suffolk and Norwich that tuberculin-testing with bovine and human P.P.D. given by the Heaf technique will reveal a pattern of reaction which is related to the incidence of human and bovine mycobacterial infection in the area.

Summary

The results of tuberculin-testing 8,839 schoolchildren simultaneously with bovine and human P.P.D. (Weybridge), using the Heaf multiple-puncture technique, in seven districts is reported. In two districts known to have had a supply of milk infected with tubercle bacilli and in which there has since been a high incidence of non-respiratory tuberculosis more reactors to bovine P.P.D. have been found than to human P.P.D., and the bovine reactions are larger than those to human P.P.D. In two towns with a known clean milk supply and a low incidence of non-respiratory tuberculosis there are more reactors to human P.P.D., and these reactions are larger than those to the bovine P.P.D. This evidence suggests that comparative tuberculin-testing of human subjects with the bovine and the human P.P.D. used in this investigation may differentiate human and bovine

TABLE VII.—*Accelerated Reaction to Specific B.C.G. Allergen and Delayed Response to Heterologous Tuberculin P.P.D. (Weybridge) Given by the Heaf and Mantoux Techniques in 30 Subjects with no Naturally Acquired Hypersensitivity who Received a Small Dose of B.C.G. Vaccine*

Test Allergen	Initial Test Reaction 72 Hours and 7 Days	Subsequent Test at 6 Months	
		72 Hours	7 Days
Mantoux 10 T.U. O.T. . .	0	5	6
M.P. P.P.D. 2.0 mg./ml. .	0	4	9
M.P. B.C.G. 20 mg./ml. . .	0	16	17

sources of mycobacterial infections. It has also been shown that there is a qualitative difference between these two P.P.D. preparations as well as a quantitative difference.

Evidence is presented suggesting that as well as an increase in the incidence of tuberculin sensitivity with age there is also an increase in size or intensity of reactions with age.

There is also evidence from the material presented and from other sources that type-specific P.P.D.s give early intense reactions (24 hours) whereas reactions to a heterologous P.P.D. are delayed, reaching their greatest intensity at 72 hours or later.

Limitations of our knowledge of the materials and the technique of comparative tuberculin-testing of human subjects are reviewed.

Other aspects of comparative tuberculin-testing requiring fuller investigation are mentioned.

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REFERENCES

- British Tuberculosis Association (1957). *Tubercle (Lond.)*, **38**, 99.
- (1959). *Ibid.*, **40**, 317.
- Carpenter, R. G., and Stewart, C. J. (1959). *Ibid.*, **40**, 251.
- Cobbett, L., and Griffith, A. B. (1913). Royal Commission on Tuberculosis (Human and Animal). Final Report. Part II, Appendix. Suppl. Vol., Report on Tuberculin Tests. Quoted by J. Francis, *Bovine Tuberculosis*, p. 142. Staples Press, London, 1947.
- Davies, J. V. (1955). *Tubercle (Lond.)*, **36**, 69.
- Edwards, L. B., Edwards, P. Q., and Palmer, C. E. (1959). *Acta tuberc. scand.*, **47**, Suppl. p. 77.
- Lewis, I., and Palmer, C. E. (1948). *Publ. Hlth Rep. (Wash.)*, **63**, 1569.
- and Palmer, C. E. (1958). *Amer. J. Hyg.*, **68**, 213.
- and Magnus, K. (1953). *Wld Hlth Org. Monogr. Ser.*, No. 12, p. 32.
- Edwards, P. Q., and Edwards, L. B. (1960). *Amer. Rev. resp. Dis.*, **81**, No. 1, part. 2.
- Enell, H. (1955). *Acta paediat. (Uppsala)*, **44**, Suppl. p. 101.
- Fenger, E. P. K., Mariette, E. S., Hutchinson, D. W., and Ouellette, A. J. (1934). *Amer. Rev. Tuberc.*, **30**, 329.
- Francis, J. (1958). *Tuberculosis in Animals and Man*. Cassell, London.
- Gedde-Dahl, T. (1952). *Amer. J. Hyg.*, **56**, 139.
- Green, H. H. (1946). *Vet. J.*, **102**, 267.
- Hagberg, E. (1950). *Acta paediat. (Stockh.)*, **39**, 461.
- Kuper, S. W. A. (1958). *Tubercle (Lond.)*, **39**, 380.
- Lind, P., and Holm, J. (1943). *Acta tuberc. scand.*, **17**, 237.
- Lorber, J. (1957). *Arch. Dis. Childh.*, **32**, 441.
- McCarter, J., Getz, H. R., and Stiehm, R. H. (1938). *Amer. J. med. Sci.*, **195**, 479.
- MacDougall, A., Mikhail, J. R., and Tattersall, W. H. (1953). *Brit. med. J.*, **1**, 64.
- Palmer, C. E., and Edwards, P. Q. (1955). *Bull. Wld Hlth Org.*, **12**, 85.
- Paterson, A. B. (1948). *J. comp. Path.*, **58**, 302.
- Pollock, T. M., Sutherland, I., and Hart, P. D'A. (1959). *Tubercle (Lond.)*, **40**, 336.
- Stewart, C. J., and Carpenter, R. G. (1961). The Measurement of Heaf M.P. Reactions. To be published.
- and McCauley, P. (1958). *Tubercle (Lond.)*, **39**, 143.
- Svenkerud, R. (1955). A Study of Heat-concentrated Synthetic Medium Tuberculin, Copenhagen.
- Tarshis, M. S., and Frisch, A. W. (1952). *Amer. Rev. Tuberc.*, **65**, 302.
- Tattersall, W. H. (1952). *Lancet*, **1**, 202.
- van Zwanenberg, D. F. (1960). *Tubercle (Lond.)*, **41**, 171.
- Stewart, C. J., Harding, K. M., and Gray, S. T. G. (1956). *Brit. med. J.*, **1**, 1464.
- Waalder, H., Guld, J., Magnus, K., and Magnusson, M. (1958). *Bull. Wld Hlth Org.*, **19**, 783.
- Wilson, G. S., Blacklock, J. W. S., and Reilly, L. V. (1952). *Non-pulmonary Tuberculosis of Bovine Origin in Great Britain and Northern Ireland*. N.A.P.T., Lond.

PEMPIDINE FOR HYPERTENSION

EXPERIENCES IN AN AFRICAN HOSPITAL

BY

K. SOMERS,* M.B., B.Ch., M.R.C.P., D.C.H.

From the Department of Medicine, Makerere College Medical School, and the Medical Division, Mulago Hospital, Kampala, Uganda

Ganglion-blocking compounds remain the sheet-anchor of successful therapy in severe hypertension. Until recently the quaternary ammonium derivatives, such as hexamethonium and pentolinium, were the only effective ganglion-blocking agents available for long-term therapy. Their major disadvantage is that they are slowly and incompletely absorbed from the gut after oral administration. Hence therapy is unpredictable and often difficult to control.

The introduction of a secondary amine, mecamlamine (Stone *et al.*, 1956), followed by a tertiary amine, pempidine (Lee *et al.*, 1958; Spinks and Young, 1958), represented a major advance in therapy. They are readily and completely absorbed from the gut (Milne *et al.*, 1957; Harington *et al.*, 1958), and are therefore satisfactory ganglion-blocking agents.

Although mecamlamine has proved very popular in the clinic management of severe hypertension (Doyle *et al.*, 1956; Smirk and McQueen, 1957; Somers and Gitau, 1959) it has several disadvantages. As its excretion is slow and irregular (Milne *et al.*, 1957), toxic effects of overdosage, particularly paralytic ileus and its sequelae, may be prolonged for several days. Patients with ileus due to mecamlamine have been unsuspectingly exposed to the risk of laparotomy (Grant and Boyd, 1957). Intestinal haemorrhage has been reported (Addison and Towers, 1959) and psychosis and incoordination, due to its accumulation within the brain, are known to occur (Schneekloth *et al.*, 1956; Harington and Kincaid-Smith, 1958; Hickish, 1958; Hift, 1958; Connor, 1958; Somers and Gitau, 1959). In contrast, pempidine has a shorter duration of action. Its excretion is more rapid, chiefly because of a lower tissue-affinity for the drug (Harington *et al.*, 1958). Toxic effects of overdosage are not likely to last as long as with mecamlamine because of the rapid elimination of the drug. Favourable reports on the long-term use of pempidine have been published by Botha (1959) and Mathisen (1959). The purpose of this communication is to describe our experience over a period of 16 months with the use of pempidine at a hypertension clinic in the middle of Africa.

Material and Methods

Sex and Age.—The series treated comprised 43 patients (18 males and 25 females). Their age distribution was 19 to 60 years (mean age 40.7 years) (Table I).

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