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Enhancing of Tuberculin Allergy by Previous Tuberculin Testing*

RAJ NARAIN,[1] S. S. NAIR,[2] G. RAMANATHA RAO,[3]
P. CHANDRASEKHAR [4] & PYARE LAL [5]

A controlled study on the enhancing of tuberculin allergy by one or more earlier tuberculin tests was carried out in a previously untested and unvaccinated population, in an area where the prevalence of non-specific allergy was known to be high. The results have shown that a test with 1 TU of PPD RT 23 in Tween 80 diluent causes subsequent tests to elicit significantly larger reactions. This enhancing effect is more marked among those with initial reactions of 8-13 mm to 1 TU or 10 mm and more to 20 TU. There is practically no enhancement among those whose reactions to 20 TU measure 0-4 mm or even 0-9 mm. Even in the group with reactions of 10 mm and more to 20 TU only some individuals show evidence of enhancement. The enhancing of allergy increases with age.

The addition of a 20 TU test increases the enhancement of allergy but mostly among those with reactions of 8-13 mm to 1 TU.

The findings suggest that enhancement may be more marked in communities with a high prevalence of non-specific allergy.

INTRODUCTION

Tuberculin tests repeated after an interval of time, at a different site, have been reported to elicit larger reactions than the first test. Magnus & Edwards (1955) suggested that intradermal testing in schoolchildren every year may prevent the waning of BCG-induced allergy. Magnus (1957) showed that waning allergy in BCG-vaccinated guinea-pigs can be substantially enhanced by an intradermal injection of tuberculin PPD in a dose of 5 TU or more and by Old Tuberculin in a dose of 1 TU. Kul Bhushan (1958-59) studied the phenomenon among vaccinated and unvaccinated tuberculin-negative and tuberculin-positive schoolchildren, and reported an enhancing of allergy among the vaccinated tuberculin-negative children. Ferebee & Mount (1963) demonstrated the enhancing effect of serial tuberculin testing in mental hospital patients. They found very little enhancement in children in four schools for the mentally retarded and thought that this lack of enhancement in the school group was due to annual testing with tuberculin. Whatever enhancement there was occurred with the first test or tests and subsequent tests added very little.

The National Tuberculosis Institute (NTI), Bangalore, is carrying out repeated surveys with tuberculin testing, X-ray and bacteriological examinations in a previously untested and unvaccinated rural population (Longitudinal Survey). In this survey persons showing reactions of 13 mm or less to 1 TU have been tested with 20 TU for a study of weak reactions. The present study, aimed at investigating a possible enhancing effect of the tuberculin test, was planned and carried out in such a way that its findings would be of assistance in interpreting the data collected in the Longitudinal Survey.

The object of the investigation was to study the possible enhancing of tuberculin allergy by previous testing with 1 TU and with both 1 TU and 20 TU.

METHODS

Owing to lack of knowledge of the factors that may influence the enhancing effect, the most suitable

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[1] Epidemiologist.
[2] Senior Statistical Officer.
[3] Junior Statistical Officer.
[4] Medical Officer, Epidemiological Section.
[5] Assistant Surgeon (Team Leader), Epidemiological Section.

size of study population could not be determined. Eight villages, in the same area as the Longitudinal Survey, were somewhat arbitrarily selected. The population, which was estimated at about 4000, had undergone no previous BCG vaccination or tuberculin testing.

All the houses in each village were numbered. A card showing the name of the village, serial number, the household number, name, age, sex and relationship to the head of the household, etc., was prepared for each individual by a house-to-house visit. All *de jure* residents, even if temporarily away and thus not likely to be available for testing, and all visitors who had slept the previous night in the village, even though they were not likely to be available at later stages of the study, were registered.

A map of the village was drawn showing the number and situation of each house, so that households could be found more easily at subsequent visits.

Round I

Before a village was visited, the cards to be used were serially numbered (individual number), and by random selection three-quarters of the cards were coded for a tuberculin test. The persons registered on these cards were each given an intradermal test on the upper third of the volar surface of the left forearm with 1 unit of tuberculin PPD RT 23 in 0.1 ml of buffered diluent containing 0.005% Tween 80. The remaining one-quarter, coded for the control group, were given 0.1 ml of the diluent containing Tween 80 and Chinosol (1 TU controls). At the time of testing, both shoulders of each individual were examined and the presence or absence of a BCG scar (definite or doubtful) was recorded, primarily to exclude any vaccinated immigrants.

Three-quarters of the individual cards coded for the 1 TU test were also coded for a further test with 20 TU. The remaining one-quarter were coded for a test with the diluent only (20 TU controls). The 20 TU tests (PPD RT 23 in 0.1 ml of the diluent with 0.005% Tween 80), or the placebo, were given at the time of reading the 1 TU test, the site being the middle of the dorsal surface of the left forearm. Only those whose reactions to the 1 TU test measured 13 mm or less (longitudinal diameter) were tested with 20 TU.

The two tuberculins and the placebos used were labelled with code marks only. None of the field workers had any knowledge of the code.

Round II

At two months, half of the registered households in each village were selected as a simple random sample in order to administer a 1 TU test to half the population. This was done irrespective of whether the particular individual in these households had been tested at Round I or not. It was considered operationally more convenient to test all persons in half the number of households rather than half the number of persons in each household. The site for retesting was the middle third of the dorsal surface of the right forearm.

Test readings

All tuberculin reactions were read after three to four days and the longitudinal diameter of the induration (in millimetres) was recorded. The knowledge of whether or not a person had been tested before was guarded from the reader. The reader and tester for the 1 TU tests were the same in the two rounds. (During the periodic assessment of the reading of tuberculin reactions carried out by the NTI, the reader was always found to be consistent in his readings.)

TABLE 1

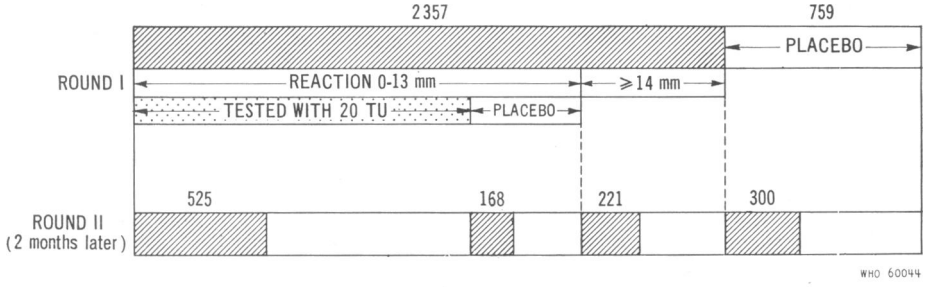
NUMBER OF PERSONS ELIGIBLE, TESTED AND READ AT ROUND I AND ROUND II

At Round I	Number of persons	At Round II [a]		
		Eligible for 1 TU	Tested	Read
For 1 TU test:				
Eligible	3 217	1 346	1 096	1 025
Tested	2 600	1 240	1 008	949
Read	2 357	1 208	976	920
For 1 TU control:				
Eligible	1 023	424	356	333
Tested	806	391	327	307
Read	759	383	319	300
For 20 TU test:				
Eligible	1 379	698	559	529
Tested	1 359	689	554	525
Read	1 275	638	513	487
For 20 TU control:				
Eligible	439	217	177	170
Tested	431	214	175	168
Read	406	201	165	158
Reactions ≥14 mm to 1 TU	539	293	240	221

[a] Excluding those registered for the first time at Round II only.

FIG. 1

DESIGN OF THE STUDY [a]



[a] Not to scale. Losses in coverages disregarded. Entire population to be retested at 18 months.

Schedule of testing

The initial 1 TU and 20 TU tests were carried out during August and September 1963, and the repeat 1 TU test, after two months, during October and November 1963. It was planned to retest the entire population with 1 TU at 18 months. The present report is based solely on the results of the first tests and those carried out two months later. (The 18-month results given in Tables 7 and 8, and Fig. 5 and 6, are those obtained in the Longitudinal Survey referred to earlier.)

Study population

The numbers of persons eligible for the various tests and for attendance at different stages of the study are shown in Table 1. Fig. 1 is a schematic representation of the design of the study. The study population comprises those tested and read at both Round I and Round II and those given the placebo at Round I but tested with 1 TU at Round II. Age and sex distributions among those tested and read at Round I, those retested and read at Round II, and the controls tested with tuberculin for the first time at Round II, did not differ greatly.

RESULTS

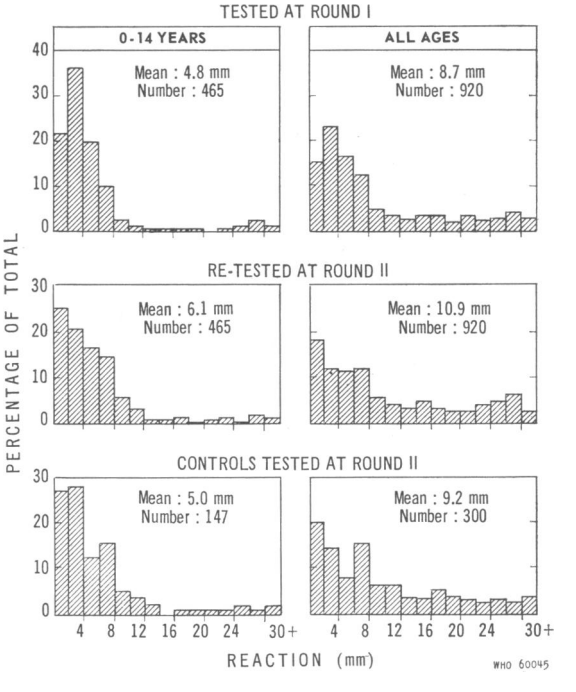
Increase in allergy seen at retest

Fig. 2 shows the distribution of indurations elicited by 1 TU for those tested and read at both Round I and Round II, and for the controls tested only at Round II. Only two age-groups—0-14 years and " all ages "—have been shown.

In these histograms and in those for the younger age-groups (which are not presented in Fig. 2) it is not possible to draw a clear line of demarcation between the " negative " and " positive " reactors. This lack of a clear separation between the two groups has been reported earlier (Raj Narain et al., 1963). The area is one with a high prevalence of non-specific allergy.

FIG. 2

COMPARISON OF REACTIONS TO 1 TU IN THOSE TESTED AT ROUND I, THOSE RETESTED AT ROUND II, AND CONTROLS TESTED AT ROUND II, FOR TWO AGE-GROUPS



The increase in mean reaction size of 2.2 mm at the retest in comparison with the results elicited by 1 TU at Round I (10.9 mm compared with 8.7 mm) may be due to many factors: new infection during the intervening two months, the effect of the time interval (including any possible reader bias) and the enhancing effect, if any, of the earlier tuberculin tests. But the increase in mean reaction size of 1.7 mm seen for retests at Round II, compared with the mean reaction to 1 TU shown by the controls tested only at Round II (10.9 mm as against 9.2 mm) can only be due to the enhancing of allergy as a result of the tests at Round I. All other factors are likely to have affected the two groups equally (see Discussion, page 631). The difference is statistically significant at the 99% level.

Some factors associated with enhancement

The effect of the time interval of two months and of factors other than enhancement (including reading and other errors) can be estimated by comparing the mean size reaction for the 1 TU controls at Round II (9.2 mm) with that for persons tested at Round I (8.7 mm). The age and sex distribution of the two groups was similar, and the difference of 0.5 mm is not statistically significant ($0.40<P<0.50$). One may therefore assume that in the retested group the difference between the reactions at Round I and those at Round II is mainly due to enhancement. A comparison of these two sets of reactions has shown that the following factors are associated with enhancement.

TABLE 2

INCREASE IN MEAN REACTION SIZE FROM ROUND I TO ROUND II, ACCORDING TO SIZE OF REACTIONS TO 1 TU AT ROUND I

Size of reaction to 1 TU at Round I (2-mm groups)	Number of subjects	Mean size of reaction to 1 TU at Round II (mm)	Increase in mean reaction size from Round I to Round II (mm)
0-1	139	4.6	4.6
2-3	207	4.5	2.1
4-5	150	6.9	2.4
6-7	111	8.4	1.9
8-9	42	13.0	4.6
10-11	28	14.5	4.1
12-13	22	17.6	5.4
14-15	25	16.4	1.7
16-17	26	16.9	0.2
18-19	15	21.5	2.9
20-21	27	22.1	1.7
22-23	19	23.7	1.3
24-25	20	25.2	0.8
26-27	30	26.3	−0.3
28-29	22	26.9	−1.6
30+	37	27.2	−5.7
Total	920	10.9	2.2

(1) *Initial reaction to 1 TU.* The increase in mean reaction size at Round II for every 2 mm of induration elicited by 1 TU at Round I, among those tested at both rounds, is shown in Table 2. The greatest increase in mean reaction size is seen for those whose reactions measured 0-1 mm and 8-13 mm at Round I. When persons whose initial reactions measure 0-1 mm are retested, a decrease in reaction size at the second test is not possible: the induration either remains the same or increases. Similarly those with reactions of 26-30 mm or more are much more likely to show only a decrease in reaction size on a retest. That such a phenomenon occurs is demonstrated in Table 3, in which only the extreme ends of the distribution of indurations at Round II, in 2-mm groups, have been compared with their mean indurations at Round I. The difference in reaction size from Round II to Round I is shown in the last column. The tuberculin reactions of 0-1 mm at Round II show an " increase " in allergy at Round I. Tuberculin reactions of 2-3 mm at Round II show the same effect, but to a very small degree. Similarly those with indurations measuring 26 mm or more at Round II show a much greater " decrease " in reaction size at Round I than those with similarly large indurations at Round I show at Round II (Table 2).

Thus the extreme upper and lower limits of Table 2 may be disregarded. The increase in reaction size is then seen to be most marked in those whose reactions to 1 TU at Round I measured 8-13 mm. The same effect continues to be noticeable, to a smaller extent, with initial indurations measuring up to about 23 mm.

A table (not presented here) similar to Table 2 was prepared by using Chauvenet's criterion to exclude extreme values (Geigy, S.A., 1956). The greater degree of increase in reaction size among those with

initial indurations of 8-13 mm was well marked. Further, the increase in reaction size at Round II could be seen even for the indurations measuring more than 23 mm.

TABLE 3

DIFFERENCE IN MEAN REACTION SIZE FROM ROUND II TO ROUND I, ACCORDING TO SIZE OF REACTIONS (REVERSE OF TABLE 2) [a]

Size of reaction at Round II (2-mm groups)	Number of subjects	Mean size of reaction at Round I (mm)	Difference in mean reaction size from Round II to Round I (mm)
0-1	165	3.0	3.0
2-3	109	2.7	0.1
4-5	106	3.7	−0.9
24-25	42	18.3	−6.3
26-27	55	21.8	−4.7
28-29	21	22.9	−5.5
30+	42	25.2	−7.5

[a] Only extreme ends of the distribution are shown.

(2) *Initial reaction to 20 TU.* A 20 TU test was given only to those whose reactions to 1 TU were 13 mm or less. The choice of this level was fairly arbitrary. The intention was to avoid large reactions to 20 TU and at the same time to test all, except those definitely positive, with 20 TU. Reactions of 14 mm and above to 1 TU were regarded as fairly certain evidence of infection with *Mycobacterium tuberculosis.*

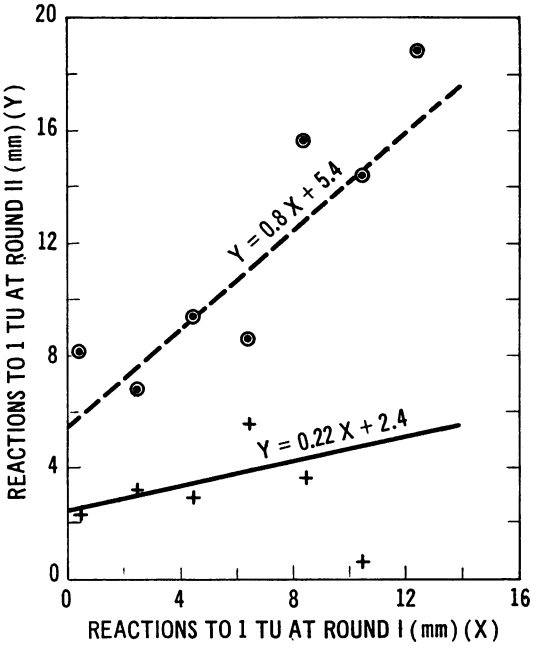
Results for the 20 TU tests are available for 487 of the 525 tested at Round I (Table 1). The increase in the size of indurations elicited by 1 TU at Round II, correlated with the size of reactions to 20 TU at Round I, is shown below in tabular form:

Size of reactions to 20 TU at Round I (mm)	*Number of subjects*	*Mean size of reaction to 1 TU at Round I (mm)*	*Increase in mean size of reaction at Round II (mm)*
0-4	102	1.7	0.6
5-9	92	3.2	0.2
10-14	83	3.1	2.3
15 and above	210	5.6	6.0
All reactions	487	3.9	3.1

The increase in allergy is seen only among those showing an initial reaction of 10 mm or more to 20 TU.[1] New infection is not likely to be more frequent among this group than among those with reactions of 0-9 mm to 20 TU. This greater increase of allergy among those with reactions of 10 mm and more to 20 TU can also be seen from Fig. 3. This figure shows the line of regression of reactions at Round II on initial 1 TU reactions separately for reactions of 0-9 mm and 10 mm or more to 20 TU. The difference in the rate of increase in allergy from Round I to Round II in the two groups is statistically significant at the 99.9% level. Further, correlation analysis shows that the reactions at Round II are more closely correlated with the

FIG. 3

REGRESSION OF REACTIONS AT ROUND II ON REACTIONS TO 1 TU AT ROUND I FOR THOSE WITH REACTIONS OF 0-9 MILLIMETRES AND 10 MILLIMETRES OR MORE TO 20 TU AT ROUND I



\+ = Mean size of reaction to 1 TU at Round II for those with reactions of 0-9 mm to 20 TU at Round I.

◉ = Mean size of reaction to 1 TU at Round II for those with reactions of 10 mm or more to 20 TU at Round I.

[1] Almost identical results were obtained when those showing reactions of 10-13 mm to 1 TU (some of whom were possibly infected with *Myco. tuberculosis*) were excluded from the table.

20 TU reactions than with the 1 TU reactions at Round I.[1]

TABLE 4

INCREASE IN MEAN SIZE OF REACTION TO 1 TU AT ROUND II IN THOSE TESTED WITH 20 TU AND THE 20 TU CONTROLS, ACCORDING TO SIZE OF REACTION TO 1 TU AT ROUND I

Size of reaction to 1 TU at Round I (2-mm groups)	Tested with 20 TU		20 TU controls	
	Number of subjects	Increase in mean reaction size at Round II (mm)	Number of subjects	Increase in mean reaction size at Round II (mm)
0-1	118	4.3	19	6.0
2-3	149	2.3	57	1.1
4-5	117	2.0	32	3.7
6-7	77	1.9	33	1.8
8-9	33	6.2	9	−1.1
10-11	15	2.9	12	6.1
12-13	16	6.6	6	2.3
All reactions (≥13 mm)	525 [a]	3.0	168 [a]	2.6

[a] Excluding four persons who refused the 20 TU test and two who refused the placebo.

(3) *Addition of the 20 TU test at Round I.* Table 4 compares the increase in size of reaction to 1 TU among those tested with 20 TU and the 20 TU controls. The increase in mean reaction size among those given both tests is 3.0 mm, while among those given only 1 TU at Round I it is 2.6 mm. The difference is not statistically significant. Thus, for the group as a whole, addition of the 20 TU test does not seem to add materially to the increase in allergy elicited by the retest. But increase in allergy due to the addition of 20 TU becomes marked if initial reactions of 8 mm or larger only are considered:

Size of reactions to 1 TU at Round I (mm)	*Increase in mean size of reaction at Round II (mm) **	
	20 TU tested	*20 TU controls*
0-7	2.7 (1.4)	2.5 (1.3)
8-13	5.4 (5.4)	2.9 (2.9)

* Figures in brackets represent increase in mean size of reaction, after excluding, by Chauvenet's criterion (Geigy, S. A., 1956), persons whose reactions have undergone extreme changes.

The numbers showing initial reactions of 8-13 mm are small, especially among the controls (Table 4). The addition of the 20 TU test seems to influence the enhancing of allergy only among those whose reactions to 1 TU at Round I measured 8-13 mm. The difference does not attain statistical significance ($0.10<P<0.20$).

To examine further the slightly greater increase in allergy among those tested with 20 TU, lines of regression of the tuberculin indurations at Round II on the initial reactions to 1 TU among those tested with 20 TU and the 20 TU controls are shown in Fig. 4. Reactions of less than 4 mm to 1 TU at Round I have been excluded because they are subject to one-sided errors, as shown in Table 3. The regression lines indicate that the two groups may behave differently. The over-all difference is not demonstrable in Table 4, because the two lines cross each other and the divergence between the two groups becomes apparent only for large initial reactions.

(4) *Influence of age.* The increase in allergy in the retested group is greater for "all ages" than for the age-group 0-14 years, whether the comparison is made with the controls tested at Round II or with the reactions obtained at Round I (Fig. 2). This suggests that the enhancing effect may be greater in the older age-groups.

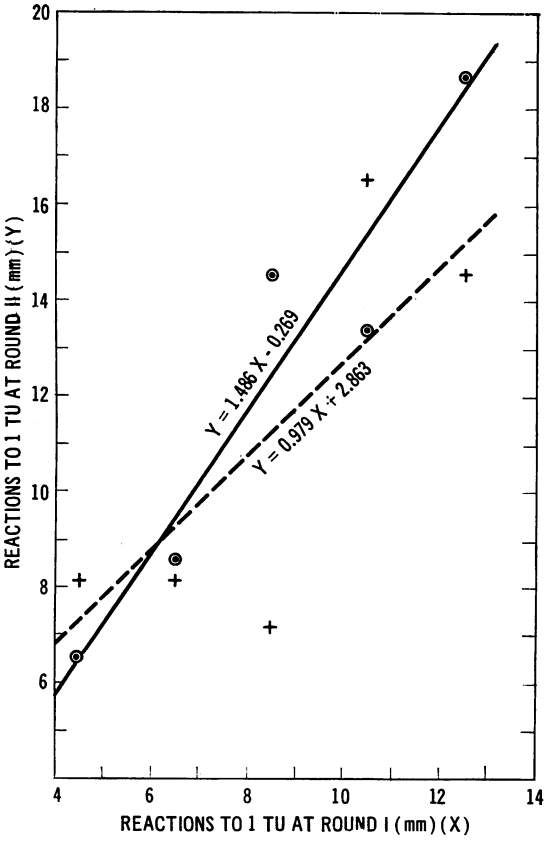
The influence of age has been studied in detail for the combined group of those tested with 20 TU and the 20 TU controls (Table 5). The enhancing of allergy at the second test is less for age-groups below 10 years, but increases with age. It is observed in persons over 45 years of age, but to a lesser degree than in those aged 25-44 years. Those tested with 20 TU and the 20 TU controls, studied separately by age-group (not tabulated), showed a similar general trend of greater enhancement of allergy with increasing age.

This phenomenon may be due, wholly or partly, to the associated increase in initial tuberculin allergy (including non-specific allergy) and to new infections. Both the last-mentioned factors increase up to a certain age and add to the increase in allergy at the second test. It may be possible, however, to

[1] The respective correlation coefficients of 0.59 and 0.45 are significantly different at the 99% level. If the reactions to 20 TU are kept constant the partial correlation coefficient between the reactions to 1 TU at Rounds I and II is only 0.22. If the reactions to 1 TU at Round I are kept constant the partial correlation coefficient between the reactions to 20 TU at Round I and those to 1 TU at Round II has a significantly higher value (0.48). This confirms that the reactions at Round II are more closely correlated with the reactions to 20 TU at Round I than with those to 1 TU at the same round.

FIG. 4

REGRESSION OF REACTIONS AT ROUND II ON REACTIONS OF 4-13 MILLIMETRES TO 1 TU AT ROUND I FOR THOSE TESTED WITH 20 TU AND THE 20 TU CONTROLS



——— = Tested with 20 TU.

◉ = Mean reaction size at Round II for those tested with 20 TU.

— — — = 20 TU controls.

\+ = Mean reaction size at Round II for the 20 TU controls.

eliminate the effect of initial allergy if those showing reactions of 0-4 mm to 20 TU are considered to have no initial allergy. As no difference in the increase in allergy by age was found between persons showing reactions of 0-4 mm to 20 TU and those showing reactions of 5-9 mm, the two have been combined in Table 6. This table shows the increase in mean size of reaction to 1 TU at Round II, by age, among those showing reactions to 20 TU of 0-9 mm and 10 mm and more. Among those with reactions of 0-9 mm,

TABLE 5

INCREASE IN MEAN SIZE OF REACTION TO 1 TU AT ROUND II IN THOSE WITH REACTIONS OF 13 MILLIMETRES OR LESS AT ROUND I, BY AGE

Age-group (years)	Number of subjects	Increase in mean reaction size at Round II
0-4	139	0.7
5-9	137	1.0
10-14	151	2.2
15-24	81	4.5
25-44	116	6.6
45+	69	4.9
All ages	693 [a]	2.9

[a] Excluding four persons who refused the 20 TU test and two who refused the placebo.

there is no difference in the increase in mean reaction size in different age-groups. Thus age, by itself, does not seem to influence the increase in tuberculin allergy among these " negative " reactors to 20 TU. It is only among those with initial reactions to 20 TU of 10 mm or more (the number of children below 5 years is only 5) that the increase in allergy is definitely greater with age.

The increase in allergy among those with reactions to 1 TU of 14 mm or more was also studied by age

TABLE 6

INCREASE IN MEAN REACTION SIZE AT ROUND II IN THOSE WITH REACTIONS OF 0-9 MILLIMETRES AND 10 MILLIMETRES AND MORE TO 20 TU AT ROUND I, BY AGE

Age-group (years)	Reactions to 20 TU measuring: 0-9 mm		≥10 mm	
	Number of subjects	Increase in mean reaction size (mm)	Number of subjects	Increase in mean reaction size (mm)
0-4	95	0.4	5	2.0
5-9	59	0.4	40	2.8
10-14	24	0.5	82	3.2
15-24			46	5.4
25-44	17	0.4	74	8.2
45+			45	4.9
All ages	195	0.4	292	5.0

TABLE 7

LONGITUDINAL SURVEY (FIRST FIFTY VILLAGES): INCREASE IN MEAN SIZE OF REACTION TO 1 TU AT 18 MONTHS, BY AGE AND SIZE OF REACTION TO 20 TU AT INITIAL TEST

Age-group (years)	Reactions to 20 TU measuring:					
	0-4 mm		5-9 mm		≥10 mm	
	Number of subjects	Increase in mean size of reaction to 1 TU (mm)	Number of subjects	Increase in mean size of reaction to 1 TU (mm)	Number of subjects	Increase in mean size of reaction to 1 TU (mm)
0-4	1 733	1.1	218	1.1	199	1.5
5-9	838	1.7	340	2.1	850	2.4
10-14	248	2.4	180	1.8	1 241	2.9
15-24	46	2.6	69	2.7	1 182	4.2
25-34	43	3.5	37	3.2	1 111	5.5
35-44	28	3.0	17	3.2	674	5.7
45+	73	3.8	46	3.4	928	6.8
All ages	3 009	1.5	907	2.0	6 185	3.8
Numbers excluded (i.e., those showing a rise of 20 mm or more)	48		14		233	

(table not presented here). No increase in allergy in successive age-groups was seen.

(5) *Influence of sex.* It was noted earlier (page 626) that the average increase in the mean size of induration at Round II due to enhancement was 1.7 mm. This average increase was greater in females (2.5 mm) than in males (0.9 mm). Among those tested with 20 TU, an increase in mean induration size of 3.4 mm for females and 2.7 mm for males was observed. The 20 TU controls showed a similar increase—3.0 mm for females and 2.1 mm for males. These differences between the sexes are not statistically significant. Among those with reactions of 14 mm or more to 1 TU at Round I, there was a decrease in mean induration size of 1.2 mm for males, but an increase of 0.5 mm for females. This difference nearly attains significance at the 95% level (P=0.055). When all the 920 persons tested and retested are considered together, the increase in mean reaction size at Round II is 1.6 mm for males and 2.7 mm for females, and this difference between the sexes is statistically significant at the 95% level.

Tables similar to Tables 5 and 6 (not presented here) were also drawn separately for the two sexes. The increase in mean reaction size was somewhat greater in females, but not consistently so in all age-groups.

On the whole, therefore, it seems likely that there is a greater enhancement of allergy in females than in males.

Supporting evidence from the Longitudinal Survey

The effect of retesting at Round II only has been studied. In order to examine further some of the relationships observed and the hypotheses formulated in the preceding section it may be relevant to examine the data from the first fifty villages in the Longitudinal Survey (Raj Narain et al., 1966[1]). Altogether 14 412 persons in the same area were tuberculin tested with 1 TU, and a further test with 20 TU was given only to those showing reactions of 13 mm or less to 1 TU. After 18 months all were retested with 1 TU. The increase in tuberculin allergy at 18 months by age and by size of reactions to 20 TU is shown in Table 7, and by size of initial reactions to 1 TU in Table 8. In order to reduce the error due to new infection, all those showing an increase of 20 mm or more have been excluded (Raj Narain et al., 1966[1]).

[1] See article on page 605 of this issue.

Table 7 shows that:

(*a*) The increase in allergy at 18 months has risen with age among those showing reactions to 20 TU of 0-4 mm, 5-9 mm and 10 mm and more. It has already been noted that no rise was observable at two months (Round II) for those with initial reactions of 0-9 mm to 20 TU (Table 6).

(*b*) The increase in allergy has risen, in practically every age-group, with the increase in size of initial reaction to 20 TU, the rise being especially marked in the group with reactions of 10 mm or more to 20 TU. Results at 18 months may not be strictly comparable with those at two months, but, broadly speaking, they seem to confirm the finding of the present report that increase in tuberculin allergy is correlated with size of initial reaction to 20 TU and with age.

Table 8 shows that the increase in allergy at 18 months does not seem to be correlated with the size of initial reactions to 1 TU. This is contrary to the findings discussed earlier (page 626). Perhaps this discrepancy is due to the fact that, on the basis of the reactions to 1 TU, it is not possible accurately to separate the " negatives " from those showing non-specific allergy. Nearly 60% of those with reactions of 0-4 mm to 1 TU showed reactions of 10 mm or more to 20 TU.

A table (not presented here) similar to Table 8, drawn separately for the two sexes, showed no difference in the enhancement of allergy in the two sexes. In view of the contrary findings described earlier (page 630), the possibly greater enhancement of allergy in females may at present be regarded as an open question.

TABLE 8

LONGITUDINAL SURVEY (FIRST FIFTY VILLAGES): INCREASE IN MEAN SIZE OF REACTION TO 1 TU AT 18 MONTHS, ACCORDING TO SIZE OF REACTION TO 1 TU AT INITIAL TEST

Size of reaction to 1 TU at initial test (2-mm groups)	Number of subjects	Increase in mean reaction size at 18 months (mm)
0-1	5 774	3.5
2-3	2 732	2.1
4-5	1 054	2.3
6-7	763	2.5
8-9	437	2.5
10-11	353	3.1
12-13	326	2.4
14-15	375	1.2
16-17	401	1.9
18-19	285	2.7
20-21	287	2.2
22-23	351	0.2
24-25	330	0.3
26-27	292	−1.3
28-29	147	−2.8
30 +	176	−3.3
All reactions	14 083 [a]	2.5

[a] Excluding 319 persons with an increase of 20 mm or more in their reactions at the initial test.

Absence of enhancement in some individuals

So far, mean induration sizes have been compared in order to study the enhancing of allergy in the group as a whole. But enhancement is not uniformly distributed throughout the group, and therefore comparison of mean induration sizes has certain limitations. This is clearly seen in Appendix Tables 1 and 2, which show the distribution of reactions to 1 TU at Rounds I and II for those whose reactions to 20 TU at Round I measured 0-9 mm and 10 mm or more, respectively. Among the former, about 80% show reactions of 5 mm or less to 1 TU at each round. There are very few (only 5%) whose reactions at Round II show an increase or decrease of more than 5 mm from their reactions at Round I (Appendix Table 1). It is only among the group with reactions of 10 mm or more to 20 TU that a substantial proportion (40%) show an increase in reaction size of more than 5 mm at Round II, while hardly any (4%) show a decrease of more than 5 mm (Appendix Table 2). The mean increase of 2.2 mm seen in the retested group at Round II (Fig. 2 and Appendix Table 3) is mainly due to the large increase in reaction size among the 40%. Thus, enhancement is mainly confined to only a part of the group showing reactions of 10 mm or more to 20 TU—that is, to an estimated 20% or less of the total population.

Among those with reactions of 10 mm or more to 20 TU, the increase in mean reaction size was practically the same in both sexes.

DISCUSSION

The present study was carried out in a previously untested and unvaccinated population, in which

only about 25% showed reactions of 14 mm or more to 1 TU and about 60% of the remainder showed reactions of 10 mm or more to 20 TU. Findings from the Longitudinal Survey confirm these results and the high prevalence of non-specific allergy in the area.

The crucial consideration is whether the enhancing of allergy discussed earlier (page 626) is really due to the tuberculin tests at Round I. A comparison of the retested and the controls tested at the same time eliminates any reader bias and the influence of other factors associated with the interval. Therefore, the difference in mean reaction size of 1.7 mm between these two groups can be due only to the effect of previous tests. It may be stressed that it is extremely unlikely—to say the least—that reader bias, or any other such factor, would produce the highly specific differences correlated with, for instance, initial allergy elicited by 1 TU and 20 TU, the number of tuberculin tests given (one or two), and age and sex (pages 626-630).[1] It is therefore concluded that previous tests do in fact enhance the allergy at retests.

The most significant finding of this paper would appear to be that enhancement caused by previous tuberculin testing, whether with 1 TU or with both 1 TU and 20 TU, is not uniform for the entire group and is associated with certain factors. When allergy at the initial test is weak or non-existent, the increase in allergy at retest is small or negligible. The enhancement is most marked among those with intermediate grades of tuberculin allergy—namely, initial reactions of 8-13 mm to 1 TU and 10 mm or more to 20 TU. Almost all those with reactions of 8-13 mm to 1 TU showed reactions of 10 mm or more to 20 TU and are therefore included in the latter group. No precise definition of the term "non-specific allergy" is available, but reactions of 10 mm or more to 20 TU (from among those showing reactions of 0-13 mm or 0-9 mm to 1 TU) may be regarded, by and large, as evidence of such allergy. This finding would then suggest that the enhancing effect of a tuberculin test may be more marked in areas, such as the present one, where the prevalence of non-specific allergy is high. It may be possible to reach a definite conclusion on this point by conducting a similar study in an area with a low prevalence of non-specific allergy.

The influence of age is somewhat difficult to interpret. Table 6 shows that, among those with reactions of less than 10 mm to 20 TU, the different age-groups show almost no increase in allergy at Round II, while among those with initial reactions of 10 mm or more to 20 TU, the increase in mean induration size is definitely greater with age. This would seem to suggest that age by itself may not be a factor, but data from an altogether different study (the Longitudinal Survey), presented in Table 7 (page 630), suggest the contrary. After an interval of 18 months, even among those with initial reactions of 0-4 mm to 20 TU, the increase in allergy rises with age. Whether or not age is an independent factor affecting enhancement, the fact remains that the increase in allergy is greater with age, and this phenomenon may be more marked in communities with a high prevalence of non-specific allergy.

The enhancement of allergy by a tuberculin test may be of the nature of an anamnestic phenomenon (Rich, 1951). The wonder is that it occurs in man with as low a dose as 1 TU of PPD. Magnus (1957) found that enhancement occurred with 5 TU, but not with 1 TU, of RT 19-20-21 in vaccinated guinea-pigs.

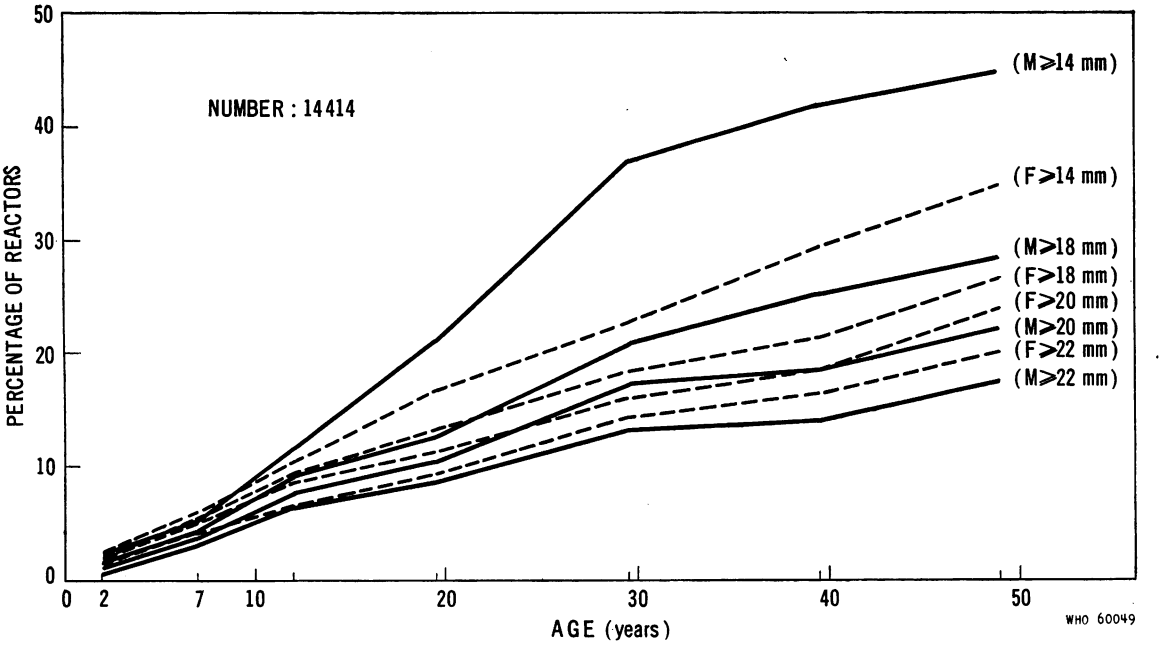
Probably, the addition of Tween 80 has made the difference, since very little tuberculin is adsorbed on the glass surfaces of syringes and containers. A tuberculin test cannot create or cause sensitivity in man. It can only increase an existing allergy, especially if this is of an intermediate grade. The enhancement of such allergy among persons vaccinated with BCG is well marked, and was first observed by Magnus & Edwards (1955). In an earlier report (Raj Narain, Kul Bhushan & Subramanian, 1961) it was found that the increase in mean reaction size elicited by retest with 1 TU after three months in previously vaccinated schoolchildren was 4.8 mm compared with 0.7 mm in unvaccinated children similarly tested. Non-specific allergy and perhaps waned allergy due to infection with *Myco. tuberculosis* are also of a similar intermediate grade, and therefore greater enhancement of allergy in the persons thus affected may not be surprising.

The report by Ferebee & Mount (1963) that there was little or no increase in allergy in schoolchildren may be commented on in passing. These authors suggest that the lack of an enhancing effect was due

[1] In three of the eight villages of the study area, during both Round I and Round II, the transverse diameters of the 1 TU reactions were recorded by another reader for a different purpose. These readings, made among the 350 persons available for analysis, also demonstrate an increase in allergy at Round II, and show a similar correlation of this increase with the size of the initial reactions to 1 TU and 20 TU. Other findings were also similar. It is extremely unlikely that a reader bias of any kind could have operated in the same way, in such great detail, with two different readers.

FIG. 5

LONGITUDINAL SURVEY (FIRST FIFTY VILLAGES): PERCENTAGE OF REACTORS AT FIRST ROUND SHOWING VARIOUS LEVELS OF REACTION, BY SEX AND AGE



to previous annual tuberculin tests. Although we have no experience with tuberculin tests at more than two points in time, the suggestion may be advanced that the schoolchildren concerned did not have much initial allergy and were possibly non-reactors to 20 TU; thus they showed no enhancing of allergy on a retest.

The enhancing effect of a previous tuberculin test has several implications:

(*a*) A far-reaching implication would be the effect on the design of future epidemiological surveys. In all surveys in which follow-up studies are planned, tuberculin testing of the entire study population may not be advisable. Only a randomly selected portion of the survey population should be tested in each round. Something similar to the design of the present study would appear to be suitable. In this way a better understanding of the changes in tuberculin status in later rounds would be possible.

(*b*) Conversion rates—i.e., the proportion of tuberculin-negative subjects who become positive—must not be used for estimates of incidence of new infections, as has often been done. This aspect has been discussed in detail elsewhere (Raj Narain et al., 1966[1]).

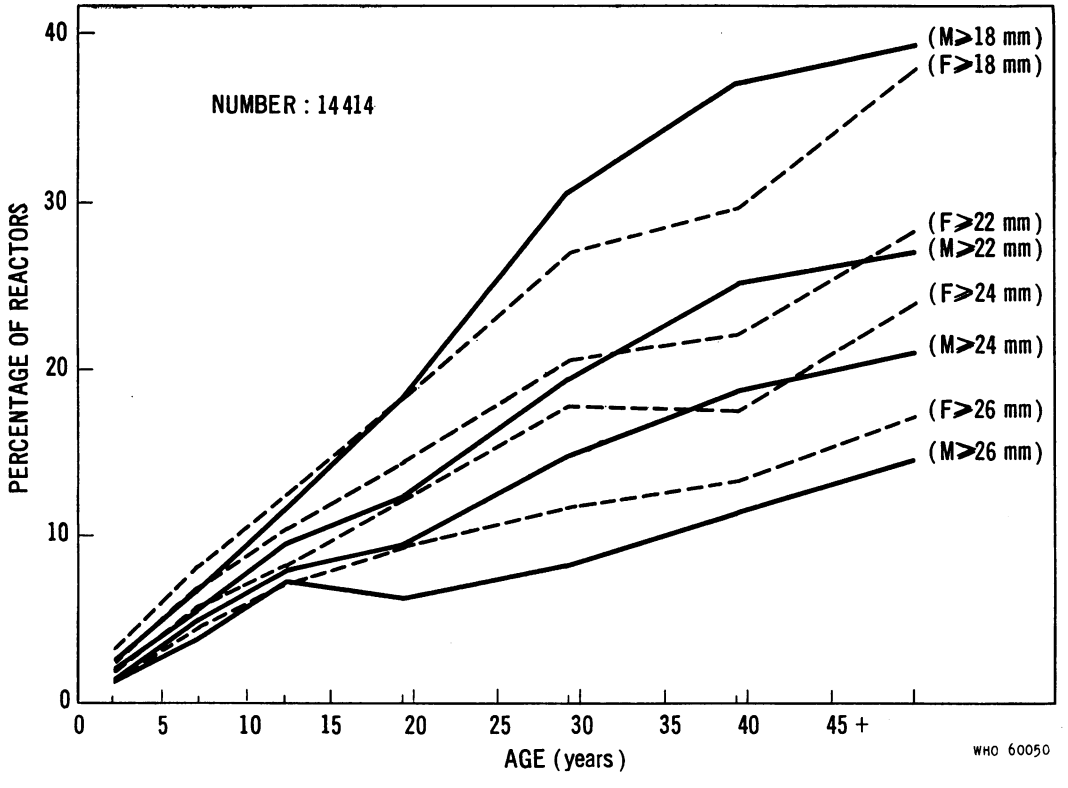
A further implication, the significance of which is known but far too often disregarded (BCG Vaccine Laboratory, Madras, 1962-63; D'Arcy Hart, Mitchell & Sutherland, 1964), is that the use of conversion rates for the measurement of post-vaccination allergy cannot be relied upon. Early reports of the efficacy of Indian BCG vaccine that were based on conversion rates (Ranganathan, 1951) proved to be misleading. It was only when measurement of the increase in mean reaction size after vaccination showed the vaccine to be a weak one that improvements were introduced leading to an allergenic potency equivalent to that of Danish vaccine (Raj Narain et al., 1961).

(*c*) In clinical practice, caution is needed in interpreting a repeat tuberculin test. For example, for diagnostic purposes a reaction of 6-8 mm to 1 TU cannot usually be regarded as definite evidence of infection with *Myco. tuberculosis*. In such cases, when the test is repeated some time later, an en-

[1] See article on page 605 of this issue.

FIG .6

LONGITUDINAL SURVEY (FIRST FIFTY VILLAGES): PERCENTAGE OF REACTORS AT EIGHTEEN MONTHS (SECOND ROUND) SHOWING VARIOUS LEVELS OF REACTION, BY SEX AND AGE



hanced reaction of 10 or 12 mm, or even larger, also cannot be taken as evidence of infection.

(*d*) Studies on the influence of steroids, chemotherapy and chemoprophylaxis on tuberculin allergy that require serial tuberculin testing of the same population could also be influenced by the enhancing effect.

(*e*) Another rather interesting effect of enhancement may be recorded. It was reported earlier (Raj Narain et al., 1963) that the percentage of reactors at the 18-mm level in different age-groups was more or less equal in the two sexes. Above this level there was a greater percentage of female reactors, and below it, there was a greater percentage of male. For the first fifty villages of the Longitudinal Survey this phenomenon is illustrated in Fig. 5 and 6 for the two rounds respectively. In Fig. 5 the percentage of reactors in the two sexes is equal at the 20-mm level. In Fig. 6 (as a result of enhancement) it is equal at the 22-24 mm level. The significance of this observation is not known.

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[1] Dr O'Rourke died on 28 July 1965.

RÉSUMÉ

Les auteurs ont étudié le renforcement de l'allergie à la tuberculine résultant d'épreuves précédentes avec 1 unité de tuberculine PPD RT23 ou 1 et 20 UT à la fois, en pratiquant au hasard ces réactions et en administrant un placebo dans la population d'environ 4000 personnes non encore testée ni vaccinée de huit villages. La prévalence de l'allergie non spécifique dans la région de l'étude était élevée. Ils ont trouvé qu'une épreuve tuberculinique renforce l'allergie mise en évidence par un test ultérieur. Il existe un rapport entre cet effet potentialisateur et le degré initial d'allergie, en particulier celui que décèle la réaction à 20 UT de tuberculine, l'augmentation s'observant presque uniquement chez ceux dont la réaction à 20 UT atteint 10 mm et plus de diamètre.

L'allergie est renforcée lorsque l'on pratique en outre une épreuve avec 20 UT de tuberculine mais surtout parmi les sujets qui présentent des réactions initiales de 8-13 mm à 1 UT. Cet effet s'accroît avec l'âge, en particulier parmi ceux dont la réaction à 20 UT de tuberculine atteint 10 mm ou plus. Il semble qu'il soit plus marqué dans les collectivités ayant une prévalence élevée d'allergie non spécifique. Les auteurs estiment que les résultats obtenus avec un matériel plus abondant devraient confirmer ceux qui ont été exposés.

Toutes les personnes d'un groupe ne présentent pas cette exagération de la réponse. Une augmentation de l'allergie de 5 mm ou plus par rapport aux réactions initiales ne se rencontre que chez 40% environ des sujets qui ont des réactions de 10 mm et plus; ils sont les principaux responsables de l'augmentation de l'allergie moyenne du groupe entier constatée lors de la seconde réaction.

Les auteurs soulignent certaines conséquences importantes de ces résultats sur la conception des enquêtes et la pratique courante d'épreuves tuberculiniques répétées.

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APPENDIX TABLE 1

CORRELATION OF REACTIONS TO 1 TU AT ROUND I AND ROUND II FOR THOSE WITH REACTIONS OF 0-9 MILLIMETRES TO 20 TU AT ROUND I

Reactions to 1 TU at Round I (mm)	Reactions to 1 TU at Round II (mm)														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
0	27	1	6	13	4	8	1	3	—	—	—	—	—	—	63
1	—	—	1	1	1	1	—	—	—	—	—	—	—	—	4
2	14	—	6	9	6	3	5	2	—	—	—	—	—	—	45
3	9	—	2	5	3	5	1	3	1	—	—	—	1	—	30
4	6	—	3	4	1	3	2	—	—	—	—	—	—	—	19
5	9	—	1	2	—	1	1	4	1	—	—	—	—	—	19
6	—	—	—	1	—	—	—	1	—	1	—	—	—	—	3
7	1	—	—	—	—	2	2	1	1	—	—	—	—	—	7
8	2	—	—	—	—	—	—	—	1	—	—	—	—	—	3
9	—	—	—	—	—	1	—	—	—	—	—	—	—	—	1
10	1	—	—	—	—	—	—	—	—	—	—	—	—	—	1
11	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
12	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
13	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total	69	1	19	35	15	24	12	14	4	1	—	—	1	—	195

APPENDIX TABLE 2

CORRELATION OF REACTIONS TO 1 TU AT ROUND I AND ROUND II FOR THOSE WITH REACTIONS OF 10 MILLIMETRES OR MORE TO 20 TU

Reactions to 1 TU at Round I (mm) \ Reactions to 1 TU at Round II (mm)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Total
0	11	—	1	1	2	1	2	3	1	1	2	—	2	1	1	3	1	3	1	1	—	—	—	1	—	—	—	1	—	—	—	40
1	2	—	—	—	—	—	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4
2	8	—	2	3	1	5	3	2	4	2	2	1	—	—	—	2	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	37
3	5	—	—	3	—	—	3	4	5	2	2	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—	—	1	27
4	10	—	—	2	1	1	—	4	5	—	4	—	—	3	1	—	2	—	1	1	1	1	—	—	—	—	—	—	—	—	—	37
5	3	—	2	1	1	3	2	4	2	—	2	1	1	—	2	—	—	1	2	1	—	—	—	—	—	2	1	—	1	—	1	33
6	2	—	—	1	2	4	1	5	2	1	—	1	—	—	—	2	—	1	—	—	—	—	—	—	—	—	—	—	—	—	1	23
7	7	—	—	3	3	3	1	3	2	—	1	—	2	—	—	3	2	1	—	1	—	—	1	1	—	1	—	1	—	—	—	36
8	—	—	—	—	1	1	1	—	2	1	—	—	1	1	—	1	—	1	1	—	—	—	—	—	1	1	1	1	—	—	—	15
9	—	—	—	—	—	1	1	—	1	—	—	—	—	—	1	1	—	2	—	1	—	—	1	—	—	—	1	—	1	—	1	12
10	1	—	—	1	—	—	—	1	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—	—	6
11	—	—	—	—	—	—	—	—	—	1	—	—	1	—	—	1	—	—	—	—	2	1	—	1	—	—	—	—	—	—	—	7
12	—	—	—	—	1	—	—	—	—	—	—	—	1	1	—	2	—	1	—	—	—	—	—	3	—	—	1	—	—	—	1	11
13	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	1	1	—	—	—	1	—	—	—	4
Total	49	—	5	15	12	19	15	26	25	8	14	3	8	6	6	15	5	11	6	5	3	2	3	8	3	5	4	4	2	—	5	292

APPENDIX TABLE 3

CORRELATION OF REACTIONS TO 1 TU AT ROUND I AND ROUND II

Reactions to 1 TU at Round II (mm)

Reactions to 1 TU at Round I (mm)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	Total
0	48	1	10	15	8	11	4	10	1	1	2	—	2	2	1	4	1	3	1	1	—	—	—	2	—	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	130
1	2	—	1	1	1	2	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	9
2	32	—	13	20	11	12	10	5	6	2	2	1	—	—	—	2	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	118
3	22	—	6	12	5	8	5	11	7	3	2	—	1	2	—	—	—	—	—	—	—	—	—	2	1	—	—	—	—	1	—	—	—	—	—	—	1	—	—	—	—	—	—	89
4	19	—	5	8	2	4	6	6	5	—	5	1	—	4	2	—	2	—	1	1	1	1	—	—	—	—	—	1	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	75
5	19	1	4	4	5	6	3	10	3	—	3	1	2	—	3	—	—	1	2	1	1	—	—	—	—	2	2	—	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—	75
6	3	—	1	3	2	5	5	13	5	4	2	1	—	2	1	2	—	1	—	—	—	—	—	1	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	52
7	9	—	1	3	3	7	3	5	4	3	3	1	3	—	—	4	2	1	—	1	—	—	1	2	1	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	59
8	3	—	—	—	1	2	2	1	4	1	—	1	2	1	—	1	—	1	1	—	—	—	—	—	1	2	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	26
9	1	—	—	—	—	2	1	—	1	—	—	—	—	—	1	2	—	3	—	1	—	—	1	—	—	—	1	—	1	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	16
10	3	—	—	1	—	1	—	1	—	—	2	1	1	—	—	1	—	—	—	—	1	1	—	—	1	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	17
11	—	—	—	—	—	—	—	—	—	1	1	—	1	1	—	1	—	—	—	—	2	1	—	2	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	11
12	—	—	—	—	1	—	1	—	—	—	—	—	2	1	—	3	2	1	—	—	1	—	—	3	—	—	1	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	17
13	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—	1	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5
14	—	—	—	—	—	—	—	1	—	—	2	—	—	—	2	—	—	—	—	—	—	—	1	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	8
15	—	—	1	—	1	1	—	—	—	—	1	—	—	—	—	3	—	1	2	1	2	1	—	—	2	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	17
16	—	—	—	—	—	—	—	1	—	1	—	1	1	—	—	2	—	—	—	—	—	—	—	—	—	1	—	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	9
17	—	—	—	—	—	—	—	—	1	—	1	1	1	—	3	1	—	1	2	—	1	1	—	1	1	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	17
18	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	1	—	—	—	—	1	—	—	1	—	1	—	1	—	—	—	—	—	—	—	—	—	—	7
19	—	—	—	—	—	—	1	—	—	—	1	—	—	—	—	1	—	—	—	—	—	—	—	3	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	8
20	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	1	—	2	—	1	2	3	1	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	15
21	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	1	—	—	—	2	—	1	2	—	—	2	2	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	12
22	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	1	—	2	2	1	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	10
23	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—	—	1	3	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	9
24	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	1	—	4	—	2	2	—	1	—	—	2	—	—	—	—	—	—	—	—	—	—	13
25	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	2	—	—	—	—	—	1	—	1	—	—	—	—	1	—	—	1	—	—	—	—	—	—	—	—	7
26	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	3	—	1	3	3	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—	13
27	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	2	—	—	1	2	2	2	2	4	—	1	—	—	—	—	—	—	—	—	—	—	17
28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1	—	1	—	1	—	1	3	1	—	—	1	—	—	—	—	—	—	—	—	—	—	1	—	—	11
29	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	1	1	1	—	—	1	1	—	—	—	1	—	1	—	1	2	—	—	—	—	—	—	—	11
30	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	1	1	3	—	1	—	—	1	—	1	—	—	—	—	—	—	—	—	—	10
31	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	2	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5
32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	1	—	1	—	1	2	—	—	—	—	1	—	—	—	—	—	—	—	7
33	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	2	—	—	—	—	—	—	—	—	—	—	—	3
34	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	3
35	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
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38	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2
39	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
40	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
41	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
42	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Total	163	2	42	67	40	61	43	64	38	16	27	9	17	13	16	28	12	16	13	13	14	11	10	25	19	23	29	26	12	9	13	4	8	6	1	3	2	2	1	—	2	—	—	920