AN INVESTIGATION INTO SPECIFIC AND NON-SPECIFIC TUBERCULIN SENSITIVITY IN SCHOOLCHILDREN IN FIFTEEN COUNTRIES

by

Dr. M.A. Bleiker

AN INVESTIGATION INTO SPECIFIC AND NON-SPECIFIC TUBERCULIN SENSITIVITY IN SCHOOLCHILDREN IN FIFTEEN COUNTRIES

by

Dr. M.A. Bleiker

AN INVESTIGATION INTO SPECIFIC AND NON-SPECIFIC TUBERCULIN SENSITIVITY IN SCHOOLCHILDREN IN FIFTEEN COUNTRIES.

Introduction.

As mortality and incidence of tuberculosis decrease in many countries, the tuberculin test is likely to become of increasing importance, primarily in estimating the infection transmission and secondly in the detection of cases of tuberculosis.

Although tuberculin sensitivity has been extensively investigated in many countries, it would be useful to compare data from several countries as to the epidemiological and clinical significance of a standard test with mammalian tuberculin.

The subject of "non-specific" tuberculin sensitivity also deserves attention in an international context. Investigators from several countries report cases of diseases resembling tuberculosis but caused by mycobacteriae other than the tubercle bacillus. It is possible that low grade tuberculin sensitivity as found in a healthy population, may be due to infection with these other mycobacterial
dia 1 organisms. Studies of the prevalence of low grade sensitivity to mammalian tuberculin in different environments and in different countries, and its relationship to sensitivity to avian tuberculin, may well contribute to our knowledge of its origin and significance.

To obtain information about the prevalence of specific and non-specific tuberculin allergy in schoolchildren in various countries, a co-ordinated international tuberculin survey is carried out under the auspices of the International Union against Tuberculosis, in co-operation with the World Health Organization.

A working protocol for this study was prepared which was approved by the Committee on Epidemiology and Statistics in September 1965.

In 19 countries groups of children of similar age have been tested intradermally with a low dose of a mammalian and an avian P.P.D. These tests were followed by high dose tests if the low dose tests showed no or only small reactions. Each of the two tuberculins is drawn from a single large batch and is administered in all countries throughout the survey.

dia 2 The present report deals with preliminary results obtained in about 50.000 children, born between 1952 and 1959, divided into 30 groups in 15 countries; in alphabetical order: Algeria, Belgium, Canada, Czechoslovakia, France, Gabon, Great Britain, Israel, Italy, The Netherlands, Poland, Portugal, Spain, Surinam and Yugoslavia.

The study was carried out during the last quarter of 1965, during 1966 and 1967.

dia 3 The following maps show the areas in the respective countries
dia 4 where the study was carried out, or is in progress, as well as the
dia 5 numbers of children tested in each area.

Selection of the groups to be tested.

The groups of children tested in each country form by no means representative samples of the school population.

The only reason for participation in this international survey was the existing possibility in the regions concerned. The kind co-operation of the respective authorities is acknowledged.

Material and methods.

The W.H.O. standard Mantoux-test was performed throughout the study. Omega Passaic tuberculin syringes were used and Eisele platinum needles.

The technicians, who injected the tuberculin and who read the tests, were all trained for a short period in the reference team of nurses, be it in the Netherlands, or in their own country. In the latter case a nurse from the Dutch reference team assisted them in starting the study.

dia 6 Two tests were given to each child simultaneously; one test was made with 2 T.U. P.P.D. Rt 23 + Tween 80, whilst the second test was made with 2 I.U. P.P.D.-avian (Weybridge '65) + Tween 80 (the so-called low dose tests). P.P.D. Rt 23 was produced in the State Serum Institute in Copenhagen, Denmark (Ir. M. Magnusson). The P.P.D.-avian was produced at the Central Veterinary Laboratory in Weybridge, England (Dr. I. Lesslie).

The high dose tests (20 T.U. P.P.D. Rt 23 + Tween 80 and 20 I.U. P.P.D.-avian Weybridge '65 + Tween 80) were given on the day of reading of the low dose tests to those children who reacted with indurations smaller than 11 mm to both low dose tests.

The reactions were read after 72 hours, a.o. by measuring and recording the transverse diameter of the indurations in mm. For each individual a separate card was filled in, which besides personalia, included information about previous B.C.G.-vaccination.

Results.

Of all results obtained, only a few will be presented in this report.

1) B.C.G.-vaccination.

The percentages of the children, who previously had been vaccinated with B.C.G. varied from country to country. Some countries have compulsory B.C.G.-vaccination, others advocate B.C.G.-vaccination on a voluntary basis; a few countries practice
dia 7 B.C.G.-vaccination on special indication only.

The next map shows the percentages of children who had been previously vaccinated with B.C.G. in the respective groups.

2) Tuberculin indices.

For the children without previous B.C.G.-vaccination in each region the percentages of those, who reacted with indurations of 6 mm and more to the low dose tests of mammalian and avian PPD were calculated.

Furthermore for each region the percentages of children who reacted with a larger induration to avian P.P.D. than the corresponding induration to human P.P.D. were calculated.

The following maps show these results.

dia 8 Map III shows the percentages of children (without previous B.C.G.-vaccination) with indurations of 6 mm and more to 2 T.U. P.P.D. Rt 23 + Tween 80.

In Great Britain regional differences are seen, ranging from 0.6 to 3.9. The overall percentage for the children tested in Great Britain is 1.5.

In the Netherlands in the city of Delft a percentage of 3.1 reactors to Rt 23 was found. In Hilversum this percentage was 2.4, whereas the percentage representative for the Netherlands in total is 1.3.

In Belgium in two small areas the percentages turned out to be 2.8 (in Louvain) and 4.1 (in Courtray).

In France the percentages of reactors to P.P.D. Rt 23, 2 Units were 9.4 in Côte d'Or (result obtained in a pilot study) and 11.7 in Gironde. (A percentage of 31.5 was found in Tourcoing.) (Gabon 42%.)

In Spain studies were made in two areas, being Alicante on the Mediterranean coast 10.0% and Castro Urdiales (Laredo), Atlantic coast 14.4.

Portugal, also two areas, the Algarve on the south coast 6.0% and in Sabugal in the central part of Portugal: 5.0%.

Sicily: Messina city: 14%; surrounding area 10%. Little island Lipari: 7%.

Crossing the Mediterranean in Algeria, three areas from north to south: coastal 39.0%, intermediate 18.3% and the southern part (dessert): 9.0%.

Further to the east: Israel with 2.5% reactors in Jerusalem (west) and 10.4 in Ramleh, an urban center between Jerusalem and Tel Aviv.

Going to the north again: Yugoslavia with respectively 48.1 and 47.4% of reactors to Rt 23 in Sarajevo and Velica Gorica (rural area near Zagreb).

In Czechoslovakia, children were tested in three areas; Prague, 54.6%, a rural area near Prague, 61.1% and a village Ivanka on Danube, near Bratislava, 54.2%.

In the south of Poland in Nowi Sacz the percentage of reactors was 29.6.

When considering the percentages in Yugoslavia, Czechoslovakia and Poland, it has to be kept in mind that these percentages were calculated from the not previously B.C.G.-vaccinated children, and that in these countries respectively about 70.95 and 50% of the children tested has been previously vaccinated.

Overseas in Surinam former Dutch Guyana the percentage of reactors was 23.9 and in Vancouver (Canada) 1.4.

e. The group of reactors to human P.P.D. in which the phenomenon of reacting with larger indurations to avian P.P.D. than to human P.P.D. is present; the non-reactors (negatives), the low grade reactors (e.g. 6-12 mm), the positive reactors (> 12 mm).

Thus it was possible to calculate for each group of non B.C.G.-vaccinated children tested, the percentage of children which reacted with indurations to avian P.P.D. which were at least 6 mm larger than the corresponding indurations to human P.P.D.

The reason we put this difference at 6 mm will be given later on.

dia 10 The next map shows in each area the percentages of children in whom the indurations to 2 U P.P.D.-avian WA'65 + Tween 80 were at least 6 mm larger than the corresponding indurations to 2 U P.P.D. Rt 23 + Tween 80.

Only in the Netherlands and in Spain this percentage is 3 or more. This may indicate that skin sensitivity to avian P.P.D. is definitely present in these areas.

Low grade sensitivity and non-specific sensitivity.

Low grade sensitivity can be defined as the phenomenon of reacting with a small induration (e.g. between 6-12 mm) to a low dose of P.P.D. (2 units).

The presence of low grade sensitivity can be demonstrated in the tuberculin histogram. This gives graphically the
dia 11 distribution of the number of indurations per mm. Low grade sensitivity is then visually demonstrated as a proportion of the histogram.

dia 12 The origin of low grade sensitivity is at least twofold:

Firstly : as the individual physiological response in t.b. infected persons to a standard tuberculin test.

Secondly: as a cross reaction evoked by e.g. human P.P.D. in a person infected with atypical mycobacteriae.

To distinguish physiological low grade sensitivity from cross reaction low grade sensitivity (or non-specific sensitivity) additional tests with sensitins prepared from e.g. avian Mycobacteriae are given in addition to the tests with human P.P.D.

As we have learned originally from the veterinarians: tests with **homologous** P.P.D. elicit indurations of wider diameter than evoked by heterologeous **P.P.D.**

Generally speaking, it may be concluded that in case the induration to human P.P.D. is larger than the corresponding induration to avian P.P.D. low grade sensitivity is considered as being physiological.

In case the induration to avian P.P.D. is larger than the corresponding induration to human P.P.D. the low grade sensitivity is considered as being non-specific.

With these rules in mind, the prevalence of non-specific tuberculin sensitivity can be estimated in the groups of non-B.C.G.-vaccinated children in each region.

First the percentage of reactors with indurations of e.g. 6-12 mm can be calculated in each group of reactors of 6 mm and more.

Secondly in this group the percentages of physiological low grade sensitivity and of non-specific sensitivity can be calculated.

Non-B.C.G.-vaccinated children tested in Delft are taken as an example.

Total number of non-B.C.G.-vaccinated boys tested: 3108. Number of indurations of 6 mm and more: 70. Of them between 6 and 12: 51. This is 73%. If from a total of positive reactors (6-24 mm) 73% is in the range of 6-12 mm, it can be stated that there is an excessive amount of low grade sensitivity. It can be supposed that this group of children is composed of physiological low grade reactors and of non-specific reactors. If it is accepted that children in which the induration to avian P.P.D. is at least 6 mm larger than the corresponding induration to human P.P.D. are to be considered as non-specific reactors, the number of physiological low grade reactors is brought back from 51 to 42, which means a reduction of 18% being the percentage of non-specific reactors.

dia 13 The results of a similar calculation for the non-B.C.G.-vaccinated children in all areas is given in the next map. Only those figures are given which are large enough to be statistically reliable, the Netherlands, France, Spain, Algeria, Poland, Surinam.

Again the difference of 6 mm and more between avian and human induration was taken as a condition for non-specificity of a given particular induration.

Non-specific low grade sensitivity is also demonstrated in the histograms.

dia 14 For comparison the histograms are given from the non-B.C.G.-vaccinated children tested in Delft (the Netherlands) and Surinam as areas with a high amount of non-specific **reactions** and from the children in Poland and Yugoslavia as areas with none or little non-specific tuberculin sensitivity.

It can be seen that in Delft and Surinam the "dip" in the histogram has gone, whilst in Poland and in Yugoslavia this "dip" is apparent.

A few words about the 6 mm difference limit between indurations to avian and to human P.P.D. in individuals in the correlation tables.

As can be seen in the correlation table of indurations to human and to avian P.P.D. in 2038 schoolgirls in the Médoc area

dia 15 in France there are three groups of children: One group is situated on the diagonal, which means that the indurations to human P.P.D. are equal in size to the corresponding indurations to avian P.P.D. The second group is on the left side of the diagonal, which indicates that the indurations to human P.P.D. are bigger than the corresponding indurations to avian P.P.D. The third group is on the right side of the diagonal, indicating that the indurations to avian P.P.D. are of wider diameter than the corresponding indurations to human P.P.D.

Let us concentrate on this last group. Is it true that all these children are not infected with human tubercle bacilli but with other anonymous mycobacteriae, which are antigenically more related to avian bacilli than to human tubercle bacilli? For, as we know from experimental studies in animals tuberculin tests with homologeous P.P.D. elicit indurations of wider diameter than heterologeous P.P.D. does.

The answer is negative: as we know the phenomenons of the cross sensitivity and of the technical errors.

Again the difference of 6 mm and more between avian and human induration was taken as a condition for non-specificity of a given particular induration.

Non-specific low grade sensitivity is also demonstrated in the histograms.

dia 14 For comparison the histograms are given from the non-B.C.G.-vaccinated children tested in Delft (the Netherlands) and Surinam as areas with a high amount of non-specific **reactions** and from the children in Poland and Yugoslavia as areas with none or little non-specific tuberculin sensitivity.

It can be seen that in Delft and Surinam the "dip" in the histogram has gone, whilst in Poland and in Yugoslavia this "dip" is apparent.

A few words about the 6 mm difference limit between indurations to avian and to human P.P.D. in individuals in the correlation tables.

As can be seen in the correlation table of indurations to human and to avian P.P.D. in 2038 schoolgirls in the Médoc area

dia 15 in France there are three groups of children: One group is situated on the diagonal, which means that the indurations to human P.P.D. are equal in size to the corresponding indurations to avian P.P.D. The second group is on the left side of the diagonal, which indicates that the indurations to human P.P.D. are bigger than the corresponding indurations to avian P.P.D. The third group is on the right side of the diagonal, indicating that the indurations to avian P.P.D. are of wider diameter than the corresponding indurations to human P.P.D.

Let us concentrate on this last group. Is it true that all these children are not infected with human tubercle bacilli but with other anonymous mycobacteriae, which are antigenically more related to avian bacilli than to human tubercle bacilli? For, as we know from experimental studies in animals tuberculin tests with homologeous P.P.D. elicit indurations of wider diameter than heterologeous P.P.D. does.

The answer is negative: as we know the phenomenons of the cross sensitivity and of the technical errors.

The question now arises which should be the smallest mm difference between an induration with wider diameter to avian P.P.D. than to human P.P.D., should groups of children be considered as suspect for an infection with other mycobacteriae than the human tubercle bacillus.

A study executed in Tourcoing in France may give some information on this problem. 195 non-vaccinated children showed zero-reactions to 2 and 20 units of P.P.D. Rt 23 and also zero-reactions to 2 and 20 units of avian P.P.D. In my opinion these children can be considered as not infected with human tubercle bacilli and not infected with any other mycobacteriae. After these tests the children were vaccinated with B.C.G. 8 weeks after the vaccination tuberculin tests were made with 2 units of human P.P.D. and with 2 units of avian P.P.D. The correlation table of the indurations shows that most of the children are situated on the left side of the diagonal,
dia 16 but also 20 others showed indurations which are of wider diameter to avian P.P.D. than to human P.P.D.

Thus it seems to be possible that persons who were infected with Bovine-B.C.G.-bacilli react with indurations of wider diameter to avian P.P.D. than to human P.P.D.

This study provided the information to put the difference between the indurations to human and to avian P.P.D. at an arbitrary limit of 6 mm.

Summary and preliminary conclusions.

1. In total 50.000 children were tested with human and avian P.P.D. in eleven countries. A uniform technique was applied throughout the study. Uniform were also syringes, needles, tuberculins and recording cards.

2. The percentages of children previously vaccinated with B.C.G. varied from region to region. No B.C.G.-vaccination was found in Spain and southern Algeria, a low percentage of vaccinees was found in Great Britain, the Netherlands, Portugal, Jerusalem and Surinam.

   A high percentage was found in Yugoslavia, Czechoslovakia and Poland and West-France.

3. The percentage of reactors to 2 U of P.P.D. Rt 23 in children without previous B.C.G.-vaccination was lowest in the Netherlands, Vancouver (Canada) and Great Britain (1.3, 1.4 and 1.5). Higher it was in Belgium (2.8 and 4.1). Still higher in France (9.4 and 11.7) and in Spain (10.0 in Alicante and 14.4 in Castro Urdiales). In Portugal the index was a little lower, 5.0 and 6.1. Still higher it was in Algeria (and Surinam) and Poland and highest in Yugoslavia and Czechoslovakia.

4. The percentages of children with reactions, which were at least 6 mm wider in diameter to avian P.P.D. than to human P.P.D., were highest in the Netherlands, in Médoc area in France, in Alicante in Spain and in Surinam.

5. Non-specific tuberculin sensitivity was found in the Netherlands, France, northern Algeria and Surinam with certainty. Probably non-specific sensitivity was found in Alicante (Spain), Jerusalem (Israel), southern Algeria. An absence of non-specific sensitivity was found in Yugoslavia and Czechoslovakia.

The study has not yet been completed and more data are coming forward.

Further analysis of the results is in progress, in which a.o. responses to low and high doses of P.P.D. will be discussed, as well as tuberculin sensitivity in previously B.C.G.-vaccinated children.

My sincere respect goes to all our co-workers in the study who without exception have been and still are helping to complete this work.

Special acknowledgement to all participating collegues for their gracious and continuous co-operation.

Thanks to Dr. Lesslie for the avian P.P.D. and to Ir. Magnusson for the human P.P.D. both products were in due time in the respective areas.

My sincere thanks to the International Union against Tuberculosis, who took this study under their auspices, to W.H.O. for their continuous stimulation and to the Organization for Health Research TNO and the Royal Netherlands T.B.-association for their material support.

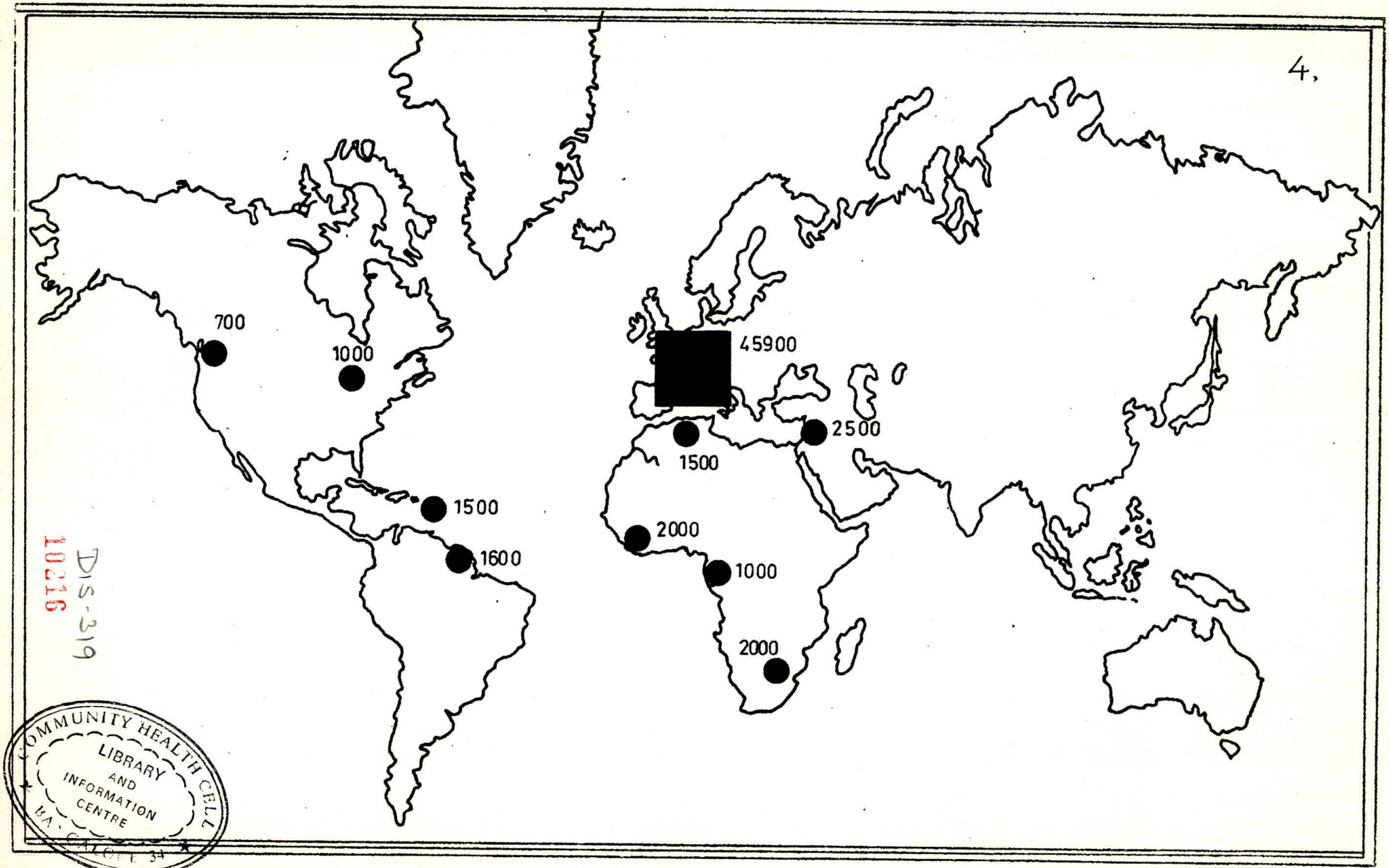
I hope that this progress report has provided some preliminary information on the prevalence of tuberculin sensitivity in comparable groups in various areas, using standard methods.
TNO. 22-9-1967. MS.

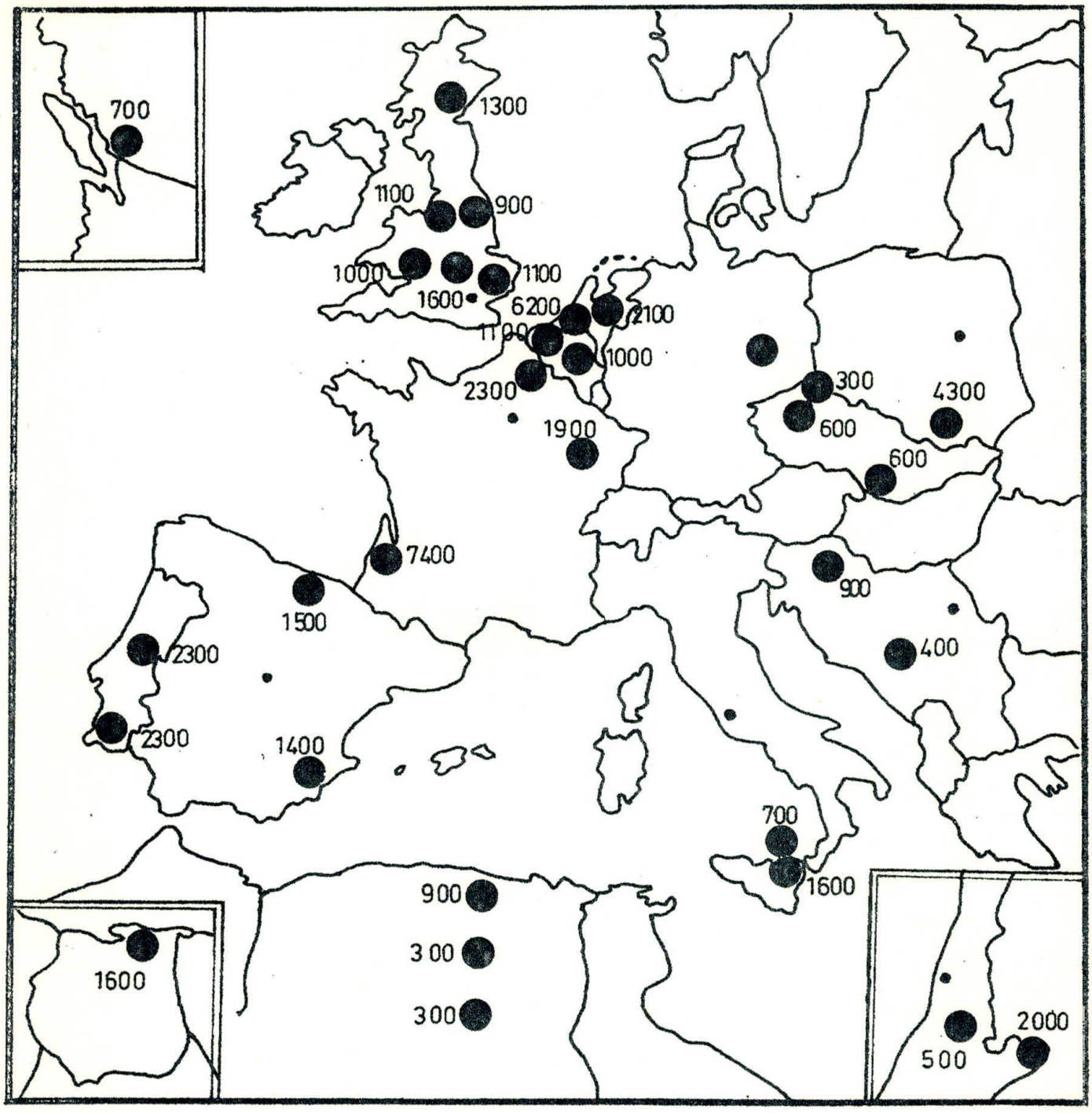
Investigation into Specific and non-specific tuberculin sensitivity in schoolchildren in 19 countries

Study population:
± 50.000 children
age : 6 _ 12 years.

Groups of children were tested in:
(alphabetical order)

- Algeria
- Belgium
- Canada
- Czechoslovakia
- France
- Gabon
- Great Britain
- Israël
- Italy
- the Netherlands
- Poland
- Portugal
- Spain
- Surinam
- Yugoslavia




700
1300
1100
900
1000
1100
1600
6200
2100
1000
2300
1900
300
4300
600
600
7400
900
1500
400
2300
2300
1400
700
1600
900
300
300
1600
2000
500

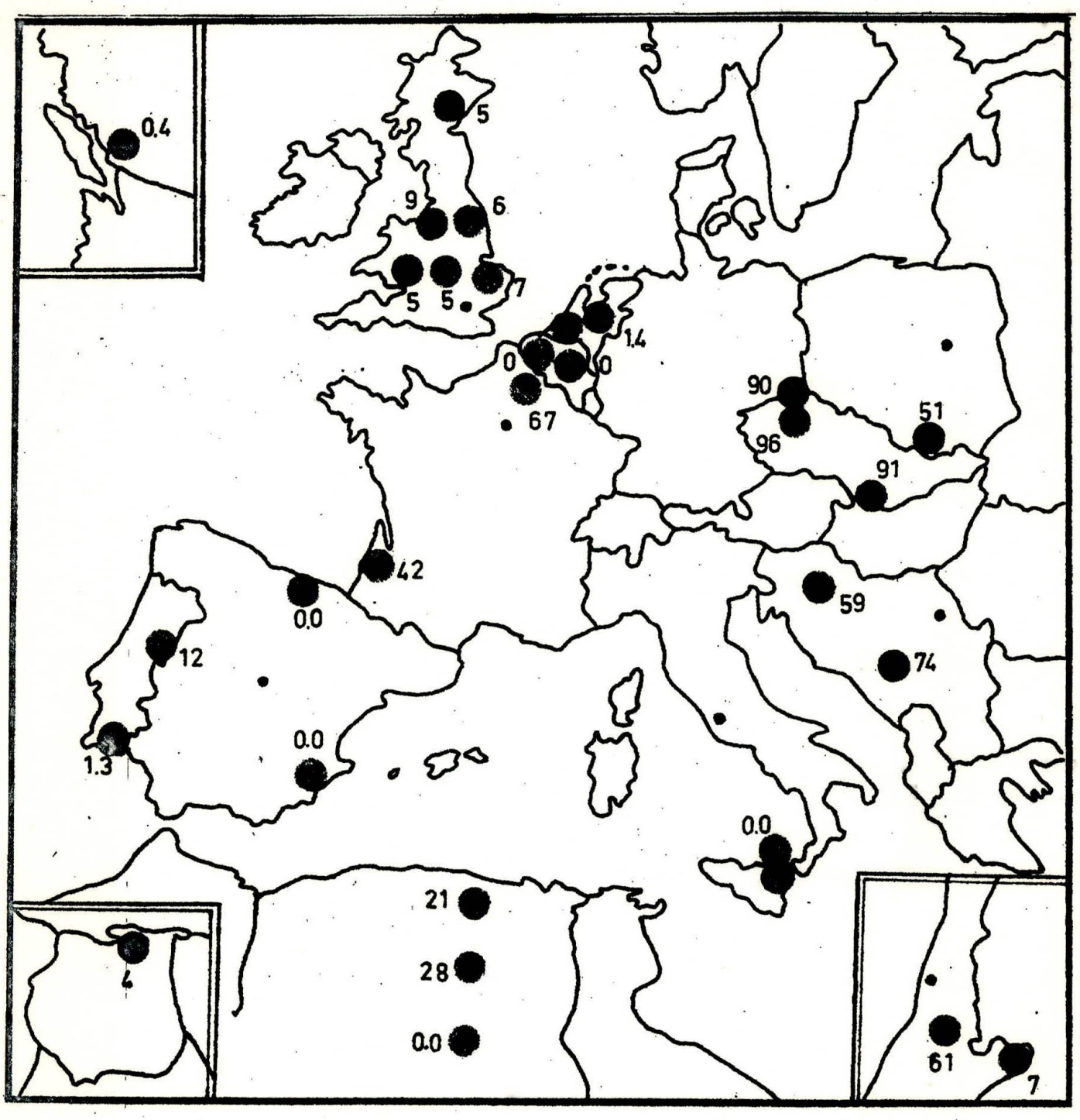
Material and methods:

W.H.O. - standard Mantoux - test

1st tests
- 2 U. P.P.D. Rt 23 + Tween 80
- 2 U. P.P.D. - avian Weybridge WA'65 + Tween 80

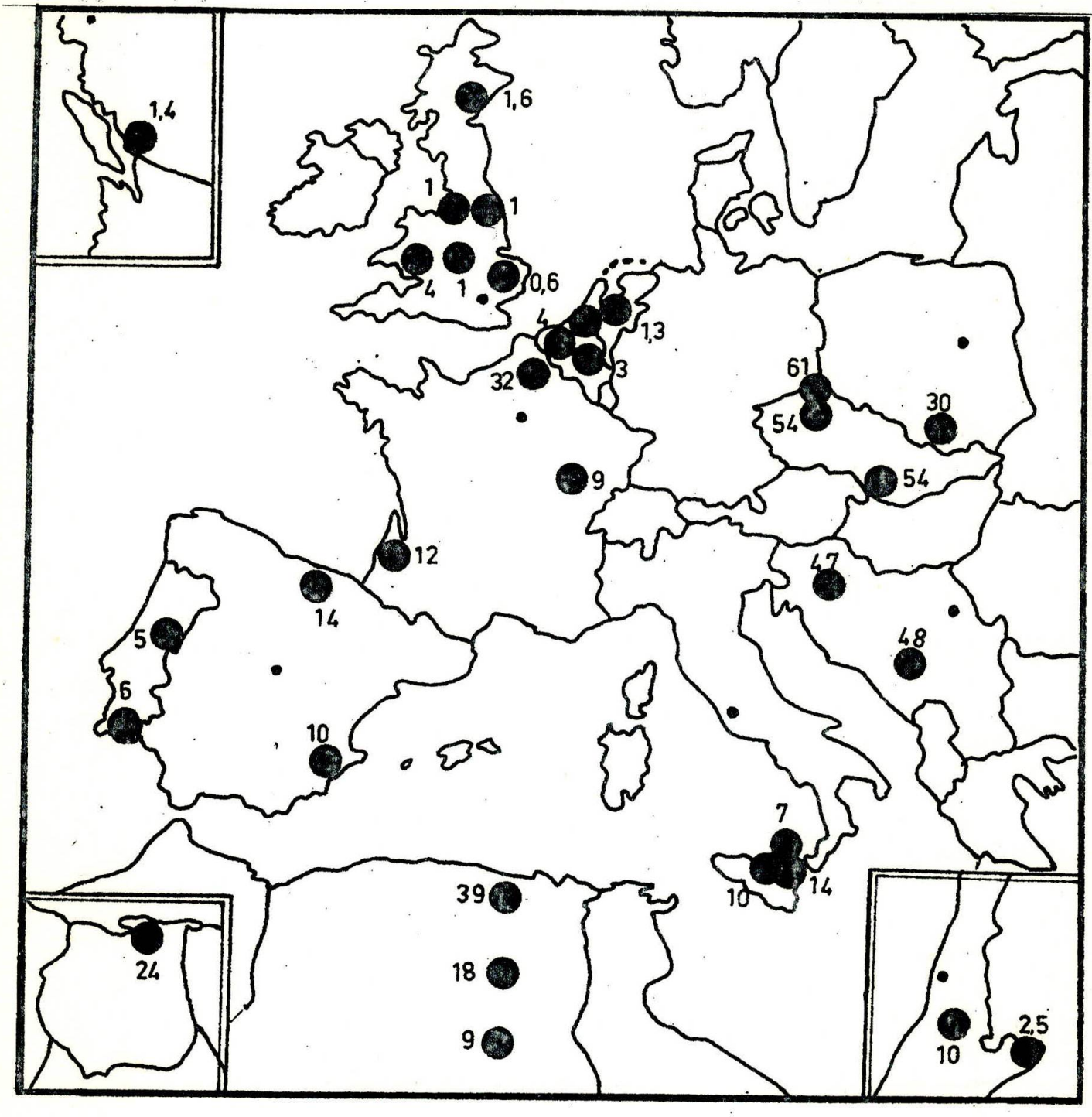
2nd tests
- 20 U. P.P.D. Rt 23 + Tween 80
- 20 U. P.P.D. - avian Weybridge WA'65 + Tween 80

Percentages of children previously vaccinated with B.C.G.

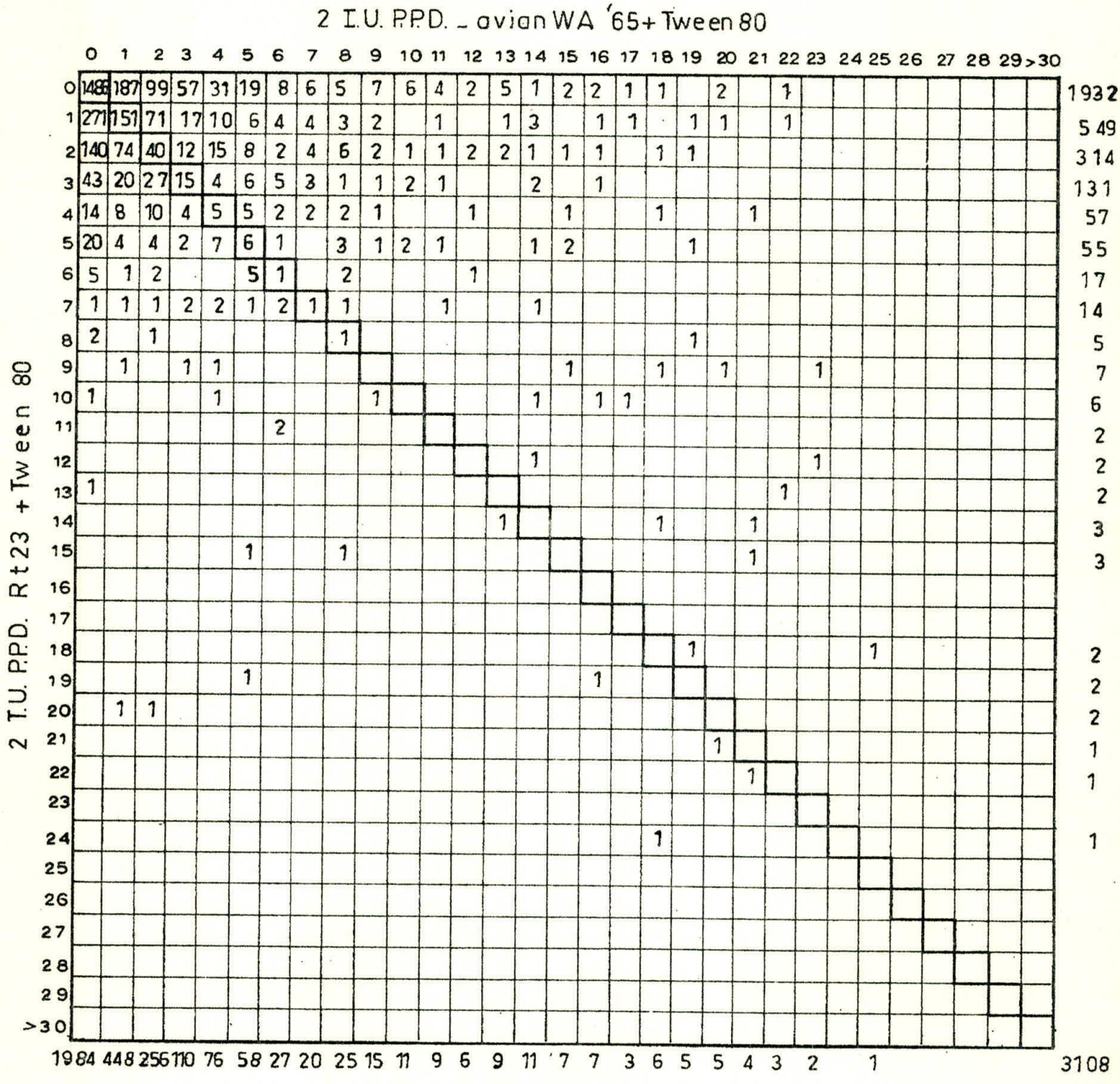


Percentage of indurations of 6 mm and more to 2 T.U. P.P.D. Rt23+Tween 80

(without B.C.G._ vaccination)

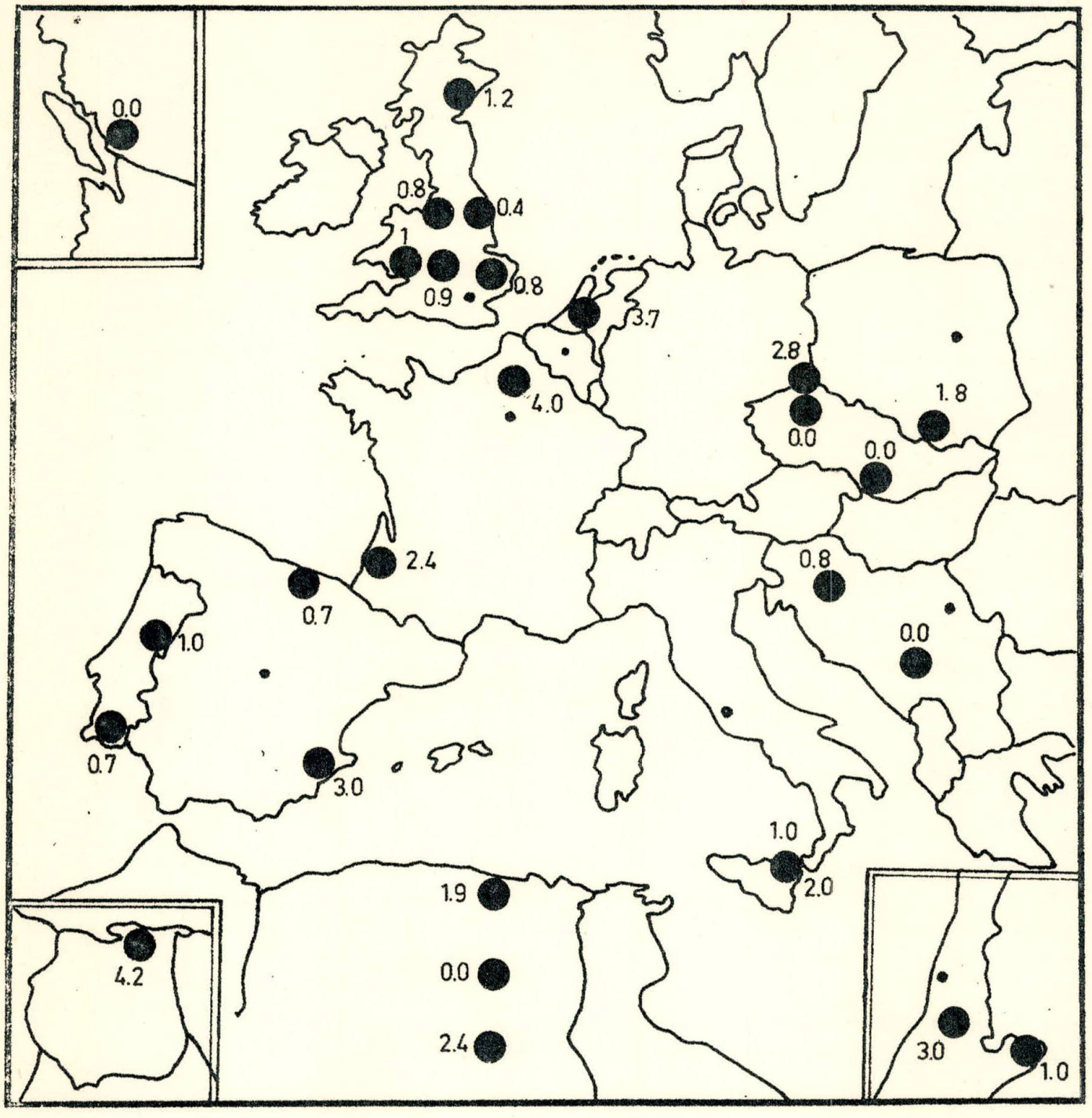


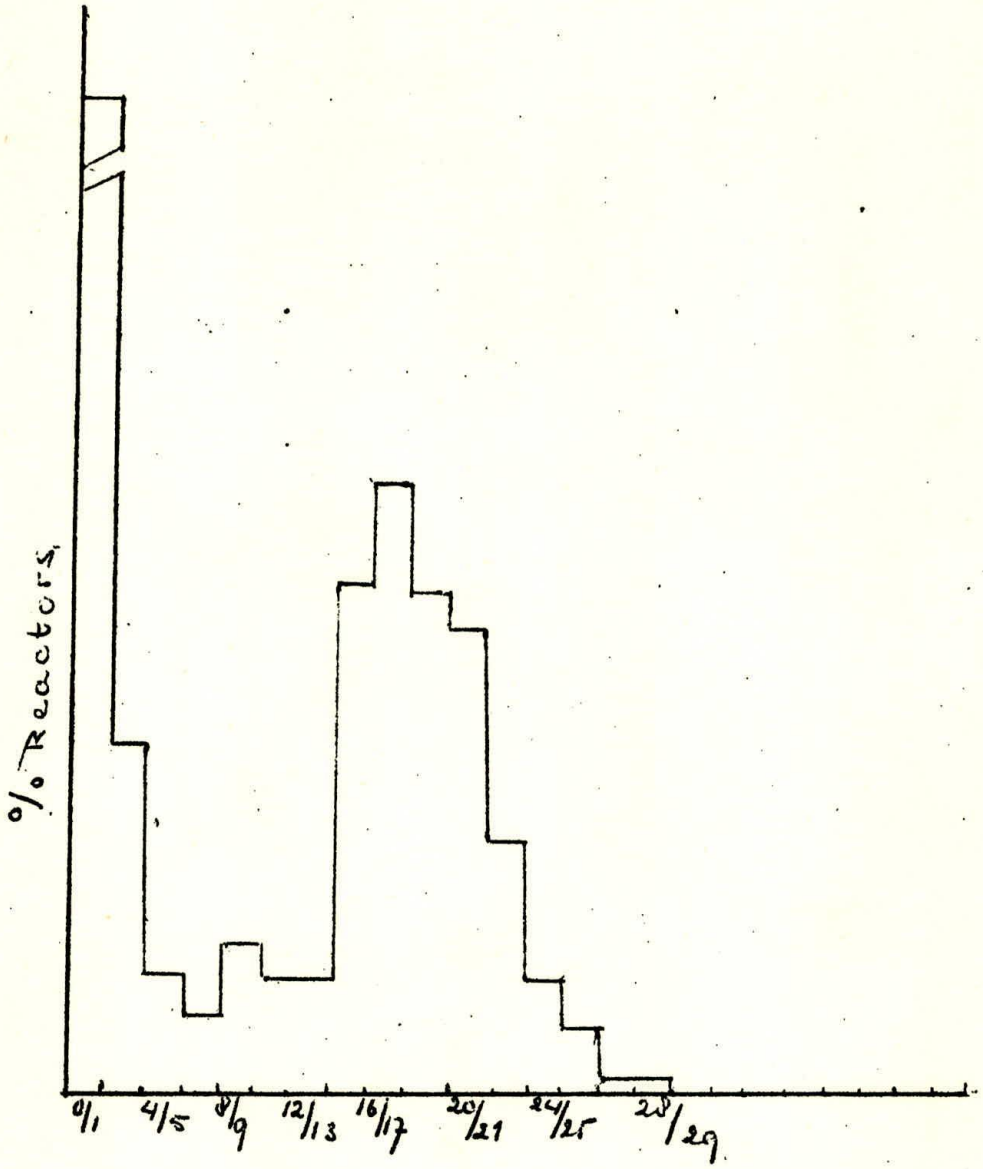
The Netherlands
Delft
boys
non B.C.G._vaccinated



6

Percentage of reactors with indurations to avian P.P.D. at least 6 mm larger than to human P.P.D.

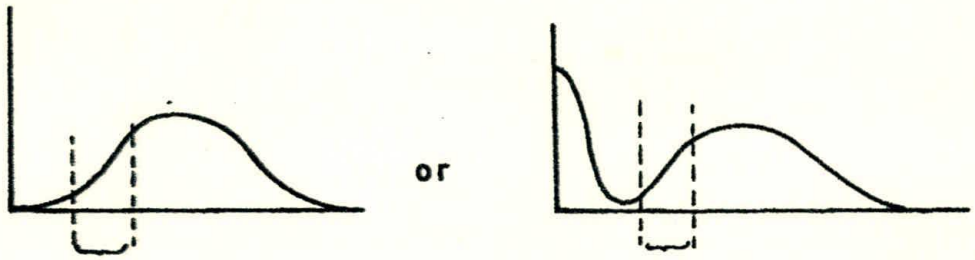



% Reactors
0/1
4/5
8/9
12/13
16/17
20/21
24/25
28/29

Origin low grode sensivity

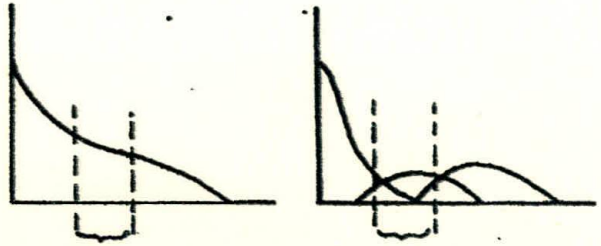
physiological low grode sensivity

a

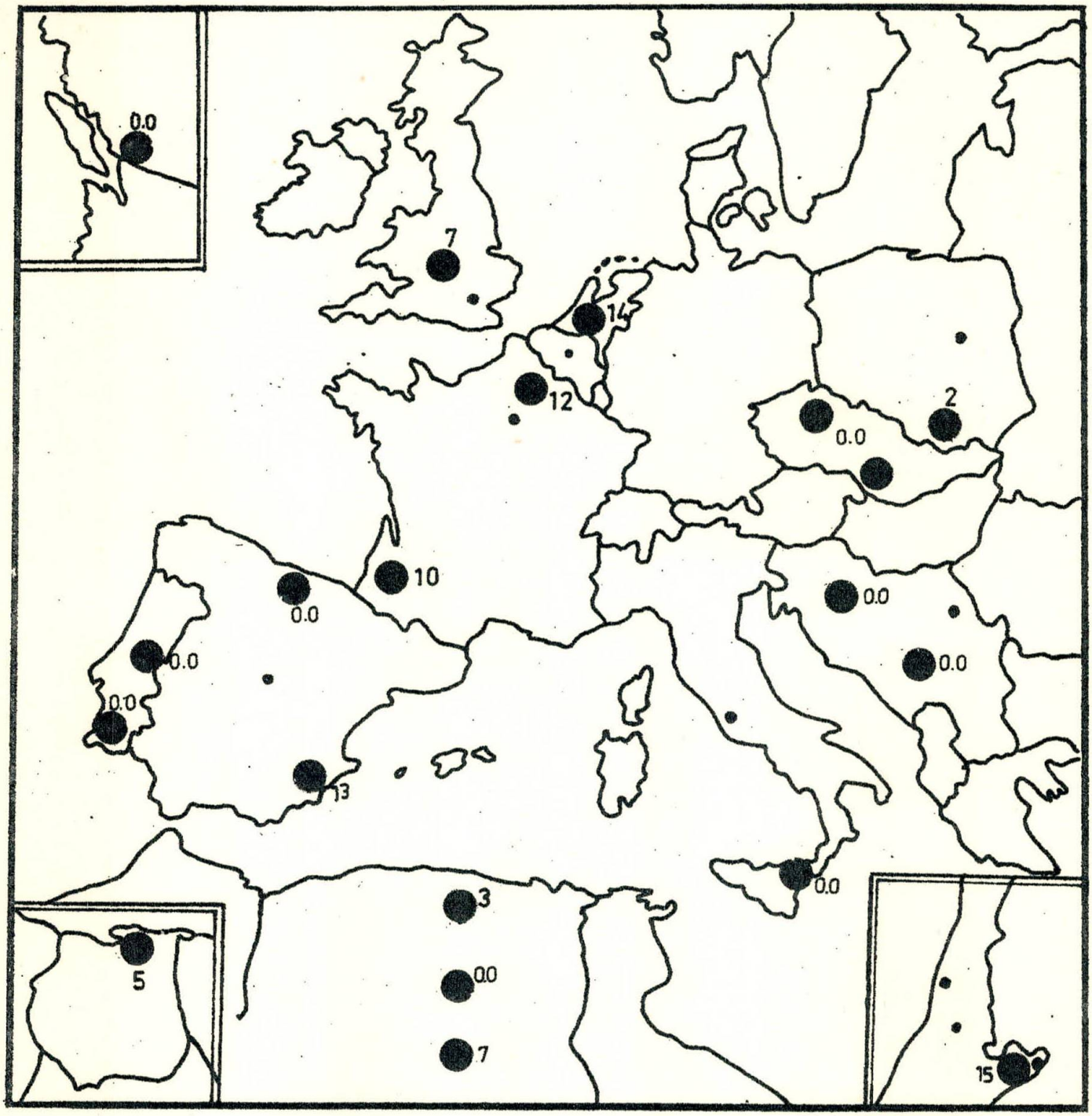


cross reaction low grode sensivity =
non specific sensivity

b



Persentage of non specific reactors to 2 T.U. P.P.D. Rt 23+Tween 80 in the range of 6_12 mm.



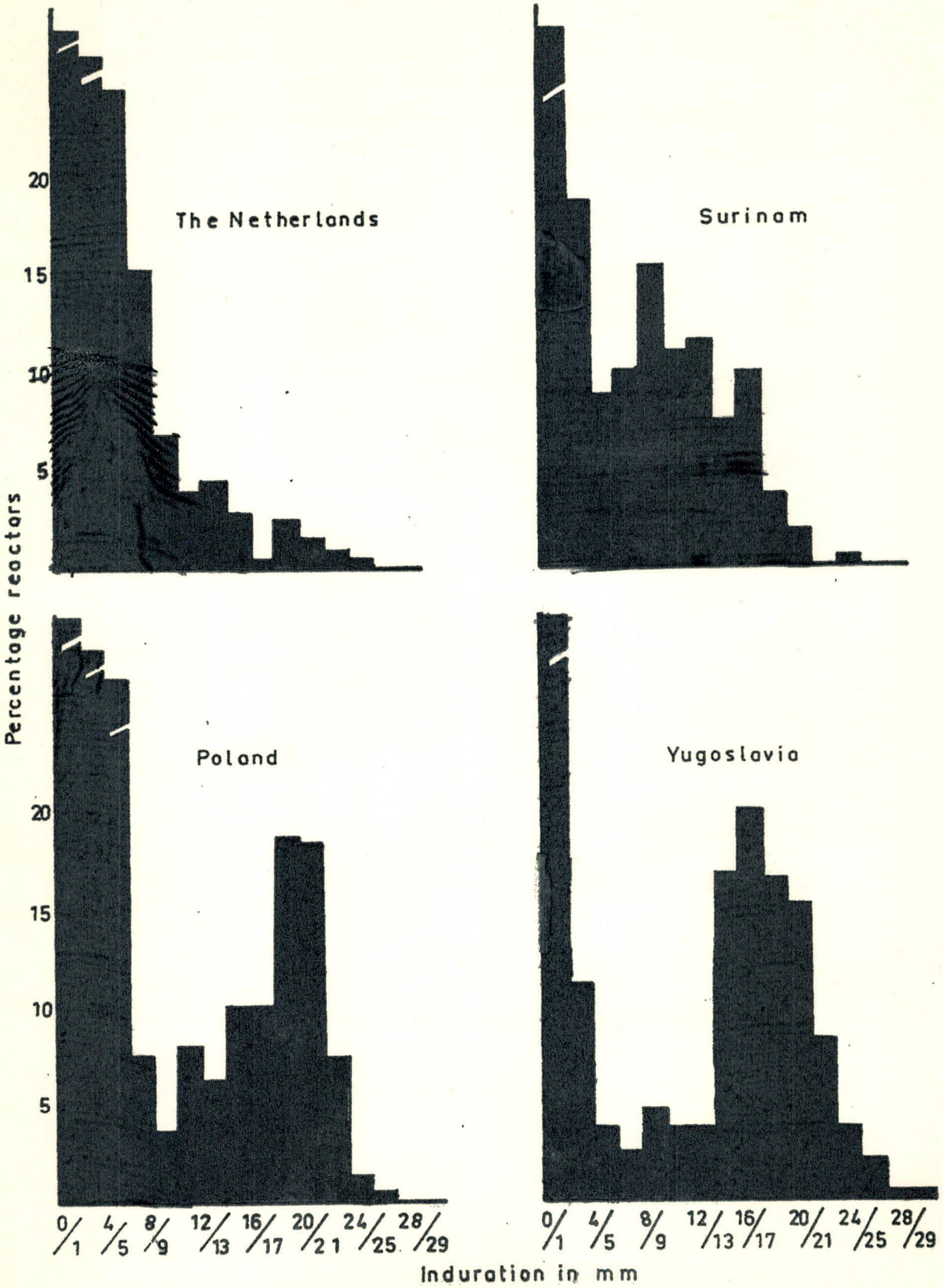
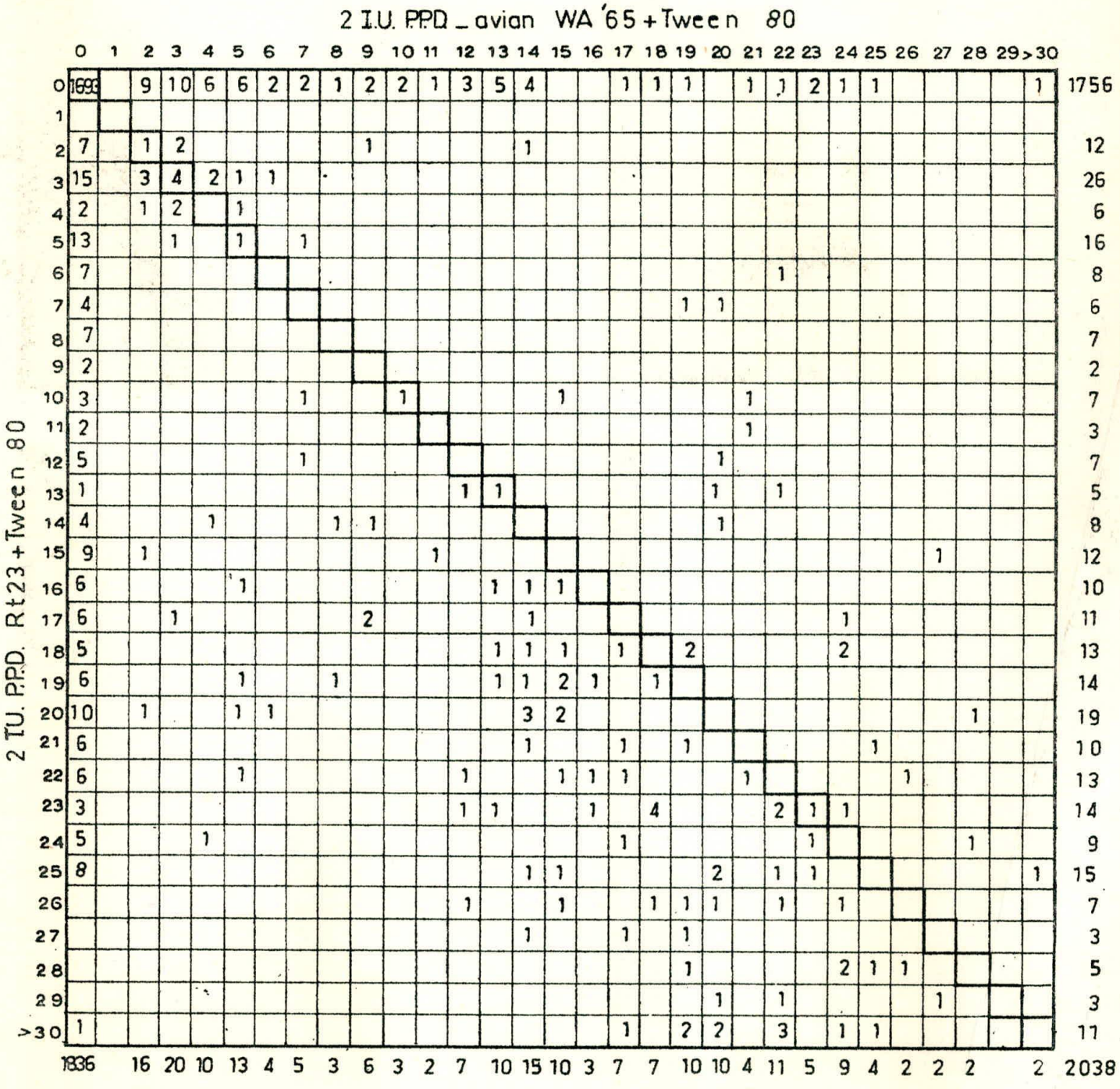
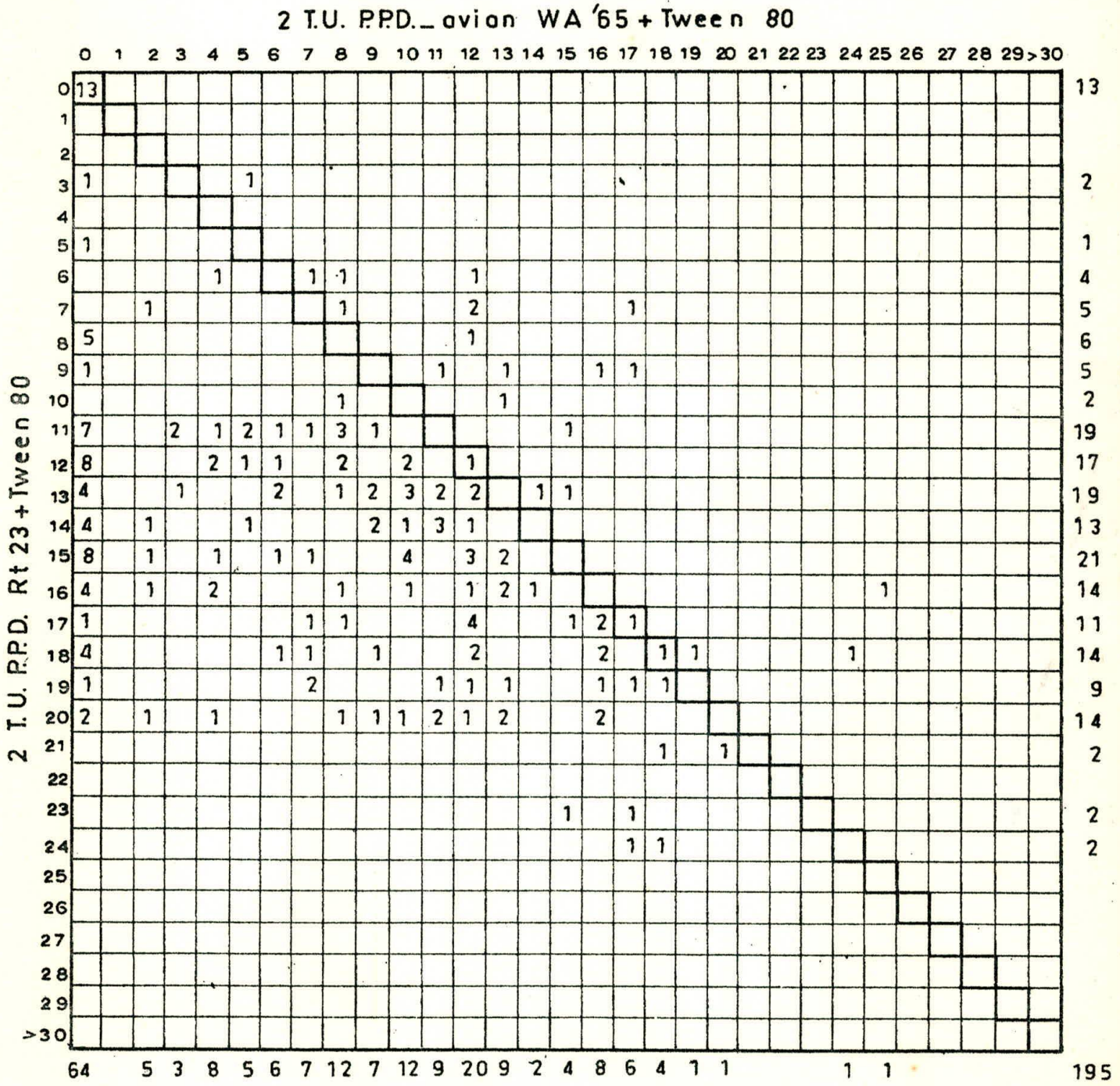

The Netherlands
Surinam
Poland
Yugoslavia
Percentage reactors
20
15
10
5
0/1
4/5
8/9
12/13
16/17
20/21
24/25
28/29
Induration in mm

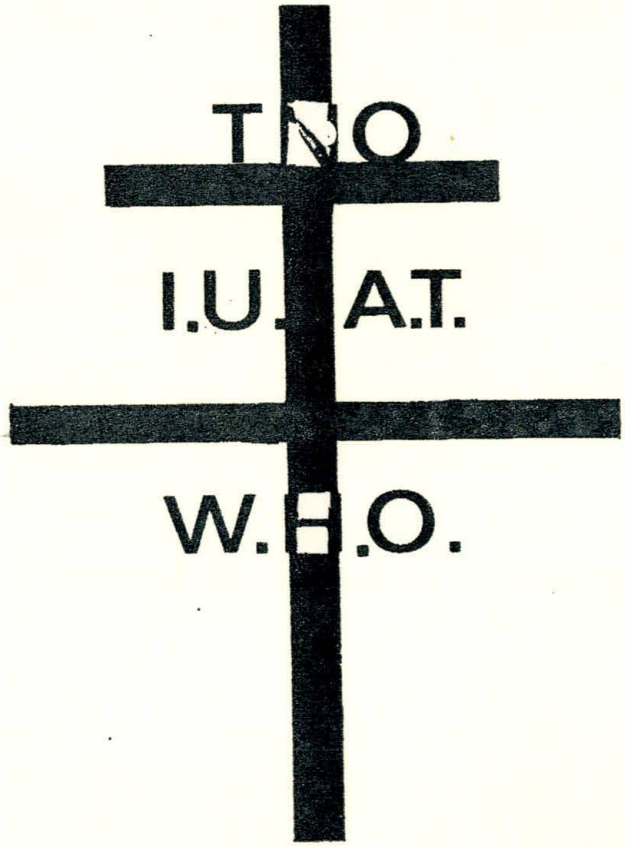
Table 13
France _ medoc
girls
non B.C.G. _ vaccinated



Tourcoing
1967



16.


T.N.O.
I.U.A.T.
W.H.O.