RUBINO'S REACTION IN LEPROSY *

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In 1926 M. C. Rubino of Montevideo, Uruguay, evolved a method of diagnosing leprosy by means of a specially prepared serum.

The procedure of preparation consisted of mixing lepers' sera together with a suspension of formalinised sheeps' red blood corpuscles in physiological salt solution. After a short time sedimentation-agglutination could be noticed, a phenomenon which occurred only when sera from lepers were used. With non-leprous serum the red blood corpuscles remained in suspension. This is known as "Rubino's reaction".

However, owing to the fact that other investigators did not obtain undeviatingly conclusive results, also that non-leprous cases occasionally gave positive reactions, Rubino perfected his technique, as noted in more recent publications.

We were anxious to try out the sedimentation-agglutination test (Rubino's reaction) for ourselves, and therefore we selected a group of subjects in whom the disease was positively confirmed, using as controls a number of normal individuals, and non-leprous cases suffering from other pathologic conditions.

History:

In 1925, Rubino, while making serological studies in syphilis, observed that one serum hastened the sedimentation of the red blood corpuscles of a sheep. Inquiry showed this serum to have been taken from a leper.

Rubino says in his most recent work [1] "As I already announced in former publications, the *reaction* evolved from an experiment designed to extract from human sera their natural hemolytic properties, so that the Bordet-Wassermann reaction could be accurately determined, without having recourse to inactivating the sera by heat in order to remove the complement.

* Received for publication June 1, 1936.

The basic idea was that red blood corpuscles of the sheep, when added to human sera, would fix their natural hemolysin, and that the sera thus sensitized would also fix the complement. Thus, the amboceptor (in this case, syphilitic antibodies) would be left free for investigation. However, as it was necessary to avoid hemolysing the sheeps' red blood corpuscles when mixed with the serum, it was imperative to fix them previously with a substance which not only fixed tissue, but did not impair their properties as an antigen. Formalin was chosen as a fixative, because it adequately preserves bacterial suspensions prepared for agglutination tests.

As previously mentioned, Rubino noted that, in the course of his investigations, in one case the corpuscles did not remain in suspension as usual, but sank to the bottom of the test tube, leaving the sera clarified. His curiosity was aroused, and on inquiry into the clinical condition of the patient, he found it to be that of a leper. Repetition of such tests brought about the same result in the majority of cases of leprosy. Rubino made his first announcement before the Society of Dermatology and Syphilology of Montevideo in June, 1926, stressing the importance only on the frequency of the phenomenon.

His first technique was as follows: He took a certain quantity of defibrinated sheeps' blood. The plasma was replaced by an equal quantity of physiological solution. The cells were washed three or four times with the same solution. Formalin, in proportion of 10 per cent to the resulting volume was added, and the mixture was left to stand for 24 hours at room temperature. Then the formalinised corpuscles were again washed three or four times with physiological solution, and the liquid replenished to its former volume with the solution. To 1 cc. of this mixture was added 1 cc. of serum, and the whole heated at 37°C for one hour.

In those test tubes containing leper serum the precipitation was rapid, being completed within an hour; in those tubes containing serum of normal individuals and of those suffering from disease other than leprosy, precipitation was slow, and did not even commence under 60 minutes.

As Rubino continued his experiments, he found that some sera taken from non-leprous subjects also produced agglutina-

tion-sedimentation, and found that when this occurred the fresh red corpuscles were also agglutinized previous to formalization, and that the phenomenon was due to the presence of a certain hetereoagglutinin which co-existed with the specific agglutinin of leprous sera. This discovery aided the perfection of the technique, and from then on he used a double series of three test tubes, each containing, respectively, increasing dilutions of serum, fresh blood formalinised corpuscles, and non-formalinised corpuscles to serve as control.

In 1928, Professors Marchoux and Caro [2], while studying Rubino's reaction, used the first technique described by the author, and obtained positive results in half the leper sera under examination, and negative results in the different sera used as control. They also varied Rubino's technique in that they mixed the leper serum and the red corpuscle suspension in proportion of 5 : 1.

Peltier [3] followed Rubino's first method and also adopted Marchoux' modification. By the first method he determined 22 per cent positive results in 18 leper sera and negative results in 75 sera of non-lepers; by the modified method he obtained 33.3 per cent positives in leper sera, also 17.3 per cent positives in the 75 sera used as controls.

Monacelli * obtained 12 positive reactions out of 13 leper sera, and found all controls negative.

In 1929, Markianos [4] published the result of his investigations in which he had found a low percentage of positive reactions, yet he admitted specificity.

Cerqueira Luz [5] obtained in 79 leper sera 50 per cent positives in cases of skin leprosy, 33.3 per cent in nerve leprosy, and 50 per cent in mixed leprosy. A group of controls, syphilitic and tuberculous, gave no positive reactions.

In 1931, Rubino [6] published an interesting treatise on his Reaction, of which he gave a full and detailed account. He made a careful study of 36 cases of leprosy in its various forms, and obtained 27 positive reactions, 7 negative, and 2 not defined. He used as control 304 sera from different cases of other illnesses, and in these latter the results were uniformly negative with one exception—that of a patient who, upon inquiry, had been found to have been in contact with lepers in a French colony, and who had both ulnar nerves greatly thickened.

* Quoted by Rubino.

Lepine, Markianos and Papayoannou [7] published their observations on 118 cases of leprosy, obtaining 50 per cent positive reactions. They consider Rubino's reaction definitely specific.

In 1932, Spanedda [8] denied all such conclusions above mentioned in as far as his own experience went, and declared himself dubious as to the specificity of the reaction. He found only one positive result in 13 leprous sera and 9 positive in 76 controls. Spanedda, however, did not explicitly follow the original technique, which may account for the unsatisfactory results he obtained.

Rubino's most recent investigations [1] made in the Bacteriological Institute of the National Department of Hygiene in the Argentine gave the following determinations:

Sera from Lepers 126	Positive 78	Negative 47	Doubtful 1
Sera from Non-lepers 39	0	39	0

Bier and Arnold [9] in a paper on serology of leprosy describe their findings in Rubino's reaction as follows: Out of 327 cases studied, 29.3 per cent were positive for nerve leprosy; 41.7 per cent for the maculo-anaesthetic type; 56.6 per cent for mixed leprosy; 66.6 per cent in nodular type and 13.8 per cent in incipient cases. Out of 945 controls they found only 0.1 per cent positive reactions.

Methods Employed by Ourselves

We strictly followed the technique of Rubino, employing his last modification in which dilutions of sera and corpuscular suspensions are registered in series of tubes.

To prepare the suspensions we used red corpuscles of sheep. We obtained the blood in quantities of 30 to 35 cubic centimetres at a time, bleeding the animal once or twice a week. The blood count of the animal was normal during the test.

To prepare the formalinised corpuscles, we placed defibrinated blood in sterile centrifuge tubes with round bases and a capacity of 50 milliliters, and noted the quantity exactly.

We washed the red blood corpuscles four times in .85 per cent physiological solution. After the last washing, the volume of water was equalized with the salt solution, which was added until it doubled the original amount of blood. Then we added slowly, stirring the while, formalin to the proportion of 10 per cent of the contents of the tubes. The preparation was left standing for 24 hours at room temperature, after which the formalinised corpuscles were again washed four times. In order to eliminate the paste-like sediment formed by the red blood cells after each centrifugation, we shook the mixture gently in order to obtain a homogeneous suspension. After the final rinsing, this suspension was washed with physiological salt solution until a red cell concentration of about 4,000,000 to the cubic mm. was obtained.

To prepare the natural red cells, we washed them in physiological saline solution, increasing the volume of the suspension until the strength of the concentration was as mentioned above.

To get the sera, we took 5 to 8 cc. of blood which we put in sterile test tubes in the refrigerator until such time as the sera had to be extracted. However, we did not leave the tubes in the refrigerator for more than 24 hours. The sera were separated under the most aseptic conditions, being put into sterile test tubes and inactivated by exposure to heat, 52–53°C, for half an hour.

The tubes and pipettes used were sterilized with great care. For each reaction we employed 6 tubes; three with formalinised blood cells and three with natural blood cells, as controls. First we put in the serum, then the physiological salt solution, and after shaking the tubes well we added the suspension of red blood cells, following this formula:

Tubes	1	2	3	4	5	6
Sera to be examined	0.5	0.25	0.1	0.5	0.25	0.1
Physiological salt solution	0.2	0.45	0.6	0.2	0.45	0.6
Suspension of formalinised red blood cells	0.1	0.1	0.1	.....	.....	.....
Suspension of normal red blood cells	.....	.....	.....	0.1	0.1	0.1

The tubes were again shaken and placed in the incubator at 37°C. Readings were made at the end of 15 minutes, 30 minutes, and again after one hour.

We classified as positive reactions those which produced agglutino-sedimentation only in the formalinised red cell series, or when it was more clearly indicated than in the natural cell series. Negatives were those series in which agglutino-sedimentation was not produced. Figure 1 shows a positive reaction; figure 2, a negative.

When agglutino-sedimentation was produced in equal quantities in both series, we absorbed the heteroagglutinins, following this technical process: For each cubic centimeter of blood we added 0.5 of a suspension of natural red cells. After being shaken, the mixture was placed in the refrigerator for an hour, during which time it was shaken three or four times at intervals. Then it was centrifuged, and the reaction antigen was prepared as follows:

Sera	0.8	0.8
Suspension of formalinised red cells	0.1	.....
Suspension of natural red cells	.....	0.1

Results

With this investigation we used Rubino's reaction with 278 sera; of these, 47 were leprous, bacteriologically confirmed; 214 were from subjects suffering from diverse pathological conditions, and 17 were from normal cases.

Sera from Lepers *

The leper blood provided us was obtained from the following sources:

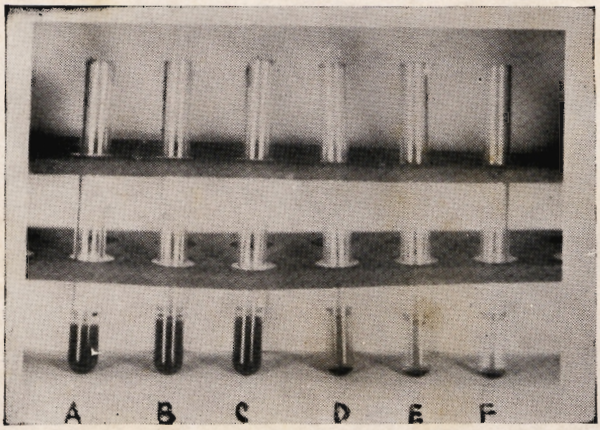
The Insular Leper Colony	11
The National Leper Colony of the Dominican Republic	34
The Dermatological Clinic of the School of Tropical Medicine (P. R.)	2

We studied a total of 47 leper sera. Of these, 33 were positive and 14 negative—i. e., 70.21 per cent gave positive reactions.

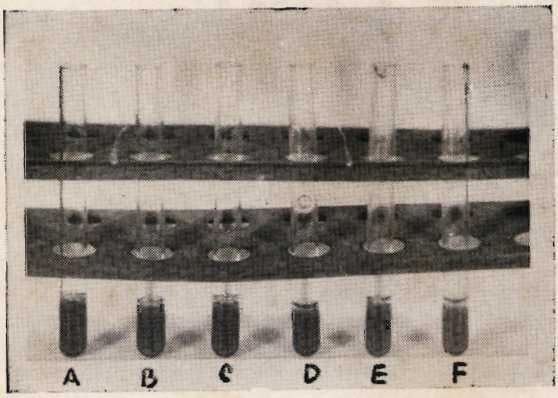
Different forms of leprosy gave the following results:

Cases	Type	Positive
19	Nodular	15
10	Mixed	7
16	Nerve	10
2	Maculo-anaesthetic	0

* Tables 3 and 4 tabulate cases studied.



Strongly positive reaction at the end of an hour.
Test tubes A, B, C, in which the red blood cells in a natural state remain in suspension.
Test tubes D, E, F, in which formalinised red blood cells show agglutino-sedimentation.



Negative reaction.
Test tubes A, B, C, with natural red blood cells, and D, E, F, with formalinised red cells in suspension.

Table 1 gives the percentage of positive cases in different types of leprosy.

TABLE 1

Type	Total	Positives	Negatives	Percentage
Nodular	19	16	3	84.21
Mixed	10	7	3	70
Nerve	16	10	6	62.5
Maculo-anaesthetic	2	0	2	0
Total	47	33	14	70.21

As may be seen by the above table, the nodular form of leprosy gives the highest percentage of positive reactions; the maculo-anaesthetic, the lowest, with two negatives in the only two cases under observation, one of whom was convalescent and showed no Hansen bacillus in the nasal mucus.

Of the cases sent from the Leper Colony of the Dominican Republic, two were almost cured, one of whom, however, still reacted to the test.

In table 2 are shown the results obtained from Rubino's Reaction by investigators in the different clinical forms of leprosy.

TABLE 2

PERCENTAGES IN THE DIFFERENT TYPES OF LEPROSY NOTED BY VARIOUS INVESTIGATORS

Names	Types of Leprosy	No. of cases	Positives	Percentages
Rubino 6	Nodular	18	16	88.88
	Mixed	10	8	80
	Nerve	8	3	37.5
Lepine	Nodular	70	49	70
Markianos and	Mixed	17	6	35.29
Papayoannou	Nerve	24	3	12.5
	Macular	7	1	14.28
Besta and	Nodular	14	4	28.57
Mariani 10	Mixed	20	18	90
	Nerve	12	2	16.66
Pimentel Imbert	Nodular	19	16	84.21
	Mixed	10	7	70
	Nerve	16	10	62.5
	Macular	2	0	00

Below we give a list of different investigators and the percentage of positive reactions they have obtained:

Names	*Percentage of Positive Reactions*
Marchoux and Caro	50
Monacelli	92.3
M. Peltier	22.22
Rubino	75
Zevallos	79
Lepine, Markianos and Papayoannou	50
Benetazzo	55
Landeiro	98
Rubino	61.9
Besta and Mariani	52.17
Pimentel Imbert	70.21

Sera Used as Controls

As controls we used 231 sera from patients suffering from different diseases, with the exception of 17 who were apparently normal. The proportions of sera used are as follows:

Patients suffering from:

Tuberculosis	55
Syphilis (Kahn + + + +) untreated	31
Syphilis (Kahn + + + +) treated	57
Carcinomas	8
Uncinariasis	11
Schistosomiasis	4
Chromoblastomycosis	2
Eczemas	6
Lupus erythematosus	4
Different dermatomycoses	7
Sprue	3
Gastro-enteritis	6
Arthritis	3
Lymphoid leukemia	1
Cirrhosis of the liver	1
Colicystitis	1
Filaria	1
Arteriosclerosis	1
Pregnancy	3
Other illnesses	9
Normal subjects	17

Of the cases of tuberculosis, 51 sera came from the Anti-tuberculosis Dispensary of San Juan, Puerto Rico. Eighty-

four from syphilitics came from the Dispensary for Venereal Diseases, Puerta de Tierra, San Juan, P. R. Three from pregnant cases were sent from the Clinic of San José, Santurce, P. R. The other sera were obtained from the University Hospital of the School of Tropical Medicine, San Juan, P. R. All these sera were negative.

Summary and Conclusions

We tested Rubino's Reaction in 278 sera: 47 from lepers, 214 from cases suffering from other conditions, and 17 from apparently normal individuals.

The agglutino-sedimentation of sheeps' red blood cells formalinised (or Rubino's reaction in its latest form) gave us a percentage of 70.21 per cent positive reactions in all cases of leprosy studied by us.

The nodular form of leprosy gave a percentage of 86.21 positive reactions; mixed leprosy, 70 per cent; nerve leprosy, 62.4 per cent. There was no reaction in non-leprous cases.

Table 3

CASES FROM THE LEPER COLONY OF SANTO DOMINGO (Dom. Rep.)

No.	Identity	Age	Sex	Race°	Form of Leprosy	Agglutino sedimentation
1	L. M. S.	30	M	M	Nodular	+++
2	L. M.	27	M	M	Nodular	+++
3	R. E. G.	31	M	M	Nodular	+++
4	J. B.	44	M	C	Nerve	+++
5	F. E.	52	M	M	Nodular	+++
6	L. A. C.	24	M	C	Mixed	—
7	M. C.	50	M	C	Mixed	+++
8	D. M.	31	M	W	Mixed	+++
9	N. U.	14	F	W	Nodular	+++
10	E. P.	65	M	C	Mixed	+++
11	J. S.	50	M	M	Mixed	—
12	S. A.	15	M	W	Nerve	—
13	R. R.	19	M	M	Mixed	—
14	M. M.	29	M	C	Mixed	+++
15	A. L.	42	M	W	Nerve°°	—
16	J. M.	24	M	M	Nodular	+++
17	M. A. F.	23	F	C	Nodular	+++
18	E. S.	33	F	M	Nodular	—
19	E. C.	39	F	M	Mixed	+++
20	J. F.	55	M	M	Nerve	+++
21	A. C.	17	F	M	Nerve	+++
22	J. S.	18	M	M	Nodular	+++
23	J. F.	50	M	M	Nerve	+++
24	L. A. T.	18	M	M	Nerve	+++
25	M. R. A.	35	F	C	Nodular	+++
26	E. M.	12	M	C	Nerve	—
27	E. M. O.	53	F	C	Mixed	+++
28	C. G.	52	F	C	Nerve	+++
29	L. F.	36	M	C	Nodular	+++
30	F. M.	35	M	M	Nerve	+++
31	C. R.	14	M	C	Nerve	—
32	R. R.	17	M	M	Nerve	+++
33	J. A. R.	24	M	M	Mixed	+++
34	F. G.	28	F	C	Nerve	+++

°M=Mulatto; W=White; C=Colored (Black)
°°Case clinically cured.

TABLE 4

CASES FROM THE LEPER COLONY OF PUERTO RICO

No.	Identity	Age	Sex	Race	Form of Leprosy	Agglutino sedimentation
1	M. S.	40	M	W	Nodular	+++
2	R. P. P.	56	M	W	Nerve	——
3	V. A.	30	M	W	Nodular	——
4	M. R.	58	M	W	Nodular	——
5	P. E.	32	M	W	Nerve	——
6	M. M.	36	M	W	Nodular	+++
7	F. C.	23	F	W	Nodular	+++
8	E. O.	23	M	W	Nodular	+++
9	E. R. L.	51	F	C	Nerve	+++
10	C. S.	33	M	W	Nodular	+++
11	R. T.	24	M	W	Nodular	+++

FROM THE DERMATOLOGICAL CLINIC OF THE SCHOOL OF TROPICAL MEDICINE SAN JUAN, P. R.

1	G. R.	58	F	W	Macular	——
2	C. G°.	36	F	M	Macular	——

°Almost cured; Bacillus of Hansen negative in nasal mucus.

Trans. C. L.

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