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Distribution of A-B-O and M-N Blood Groups among Ceylonese ; their significance in Forensic Work

by

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(With 2 Text figures and 14 Tables)

THIS is a purely factual record of the distribution of the A-B-O and M-N blood groups among the Ceylonese. The chances of exclusion in the case of a false accusation of paternity are also worked out in detail.

The distribution of blood groups among the Ceylonese has been studied by several workers. In 1937, Hill[1] published the results of an investigation into the A-B-O system as distributed among 940 persons. Seneviratne (1945)[2] grouped 4,685 persons on the A-B-O system. Koch studied the Rh groupings of 551 persons in 1952[3], and the Rh and A-B-O groupings of 524 cases in 1953[4]. The figures reported by the above will be taken up with the present figures in the discussion. As far as we are aware, there has been no published study of the M-N factors among the Ceylonese.

Methods

Tests were carried out on University students in connection with the freshmen's medical examination, and among various other groups. Recruits to the Police Training School were also examined during their routine blood grouping. In each case nationality was ascertained by questioning. Most of them were either Sinhalese or Ceylon Tamil, but there were some of mixed parentage, and these were classified as ' others ' together with Moors, Malays, Indian Tamils, Parsees and Burghers (a term originally restricted to the descendents of personnel employed by the Dutch East India Company).

The serum used was anti-M and anti-N ' crude serum ' obtained from the Behringwerke Laboratories in Germany. The actual preparation of the testing fluid was done according to the method described by Wiener (1933)[5]. The serum was diluted 30 times and absorbed by packed washed red cells. In the case of anti-N serum, two absorptions were carried out with 1/2 and 1/8 volume of packed red cells of group AM for 1 hour, and 15 minutes respectively. In the case of anti-M serum, an equal volume of AN cells was used and the absorption was carried out for 45 minutes. Difficulty was experienced in obtaining a person who was known to be of the group AN. We were assisted in this matter by Dr. R. P. V. Jayawardene, who had determined the blood groups of about 70 people at the

Medical Research Institute, Colombo, by sending the blood to the Lister Institute, London, for grouping. Of those examined there was one AN available and cells obtained from him were used in the absorption. Dilution and absorption as above gave a testing fluid of the titre of 1: 6 for Anti-M sera and 1: 4 for Anti-N sera.

The technique used for the actual tests for M-N grouping was the open slide technique. This gave rapid readings with very few doubtful cases. It was found that allowing the slide to rest for about a minute after agitation helped considerably in the reading. On examining the cells immediately after agitation it was noticed that there was a tendency for the cells to appear to be in clumps, but on sedimenting, this disappeared and there was no difficulty in separating the negatives from the positives. No case of M negative and N negative was found and the testing sera were checked at periodic intervals against the known groups in the list furnished by Dr. Jayawardene.

Results

Table I shows the results obtained in the total group of 1302 persons.

TABLE I

A-B-O and M-N groupings in Present Investigation

Number Examined	A			B			AB			O		
	M	N	MN	M	N	MN	M	N	MN	M	N	MN
Sinhalese 925	82	29	105	81	27	113	13	7	32	173	54	209
Ceylon Tamil 307	27	6	33	39	16	32	12	1	5	46	28	62
Others 70	7	1	8	11	1	7	—	—	3	8	5	19
Total 1302	116	36	146	131	44	152	25	8	40	227	87	290

Table II gives the percentages of the different groups in the A-B-O system.

TABLE II

A-B-O Percentages in Present Investigation

Number Examined	A		B		AB		O	
	No.	%	*No.*	%	*No.*	%	*No.*	%
Sinhalese 925	216	23.35	221	23.89	52	5.62	436	47.14
Ceylon Tamil 307	66	21.50	87	28.34	18	5.86	136	44.30
Others 70	16	22.86	19	27.14	3	4.29	32	45.71
Total 1302	298	22.89	327	25.12	73	5.61	604	46.39

Table III gives the percentages found in the M-N system, and the figure for χ^2 in each case (see statistical analysis.)

TABLE III

M-N Percentages in Present Investigation and χ^2

Number Examined	M		N		MN		χ^2
	No.	%	*No.*	%	*No.*	%	
Sinhalese 925	349	37.73	117	12.65	459	49.62	3.23
Ceylon Tamil 307	124	40.39	51	16.61	132	43.00	2.41
Others 70	26	37.14	7	10.00	37	52.86	1.40
Total 1302	499	38.33	175	13.44	628	48.23	1.05

Statistical Analysis

A-B-O System

A suitable statistical test for the accuracy of the investigation is given by calculating the frequencies of the genes A, B and O (called p, q and r, respectively), in the population under survey.

The following formulae are given by Wiener (1946)[6] for calculating these figures :—

$$p = 1 - \sqrt{\bar{O} + \bar{B}}$$
$$q = 1 - \sqrt{\bar{O} + \bar{A}}$$
$$r = \sqrt{\bar{O}}$$

where $\bar{A}$, $\bar{B}$ and $\bar{O}$ are respectively the frequencies of the groups A, B and O.

The figure $p+q+r$ should theoretically be unity, and the deviation in relation to the probable error for the sample gives a means of estimating the probability of the results.

The probable error is given by the formula :

$$\frac{0.6745}{\sqrt{N}} \sqrt{\frac{pq}{2(1-p)(1-q)}}$$

These figures are given in Table IV.

TABLE IV

Gene Frequencies in A-B-O System

	p	q	r	$1-(p+q+r)$	*P.E.*	*Deviation* / *P.E.*	*Probability*
Sinhalese	0.157	0.160	0.687	0.004	0.003	1.3	0.48
Ceylon Tamil	0.148	0.189	0.666	0.003	0.005	—	—
Total (including others)	0.154	0.168	0.681	0.003	0.002	1.2	0.58

M-N System

Since the first publications of the frequencies of the MN factors by Landsteiner and Levine there have been many papers published giving results of MN groupings. A number of these were collected by Taylor and Pryor, (1939)[7] and Wiener[6]. Taylor and Pryor analysed the results statistically and decided that some of the figures were unreliable. The test used was the χ^2 test of Fisher. The two gene theory put forward by Landsteiner and Levine and confirmed by subsequent workers was the foundation on which the test is based. The formula on which the χ^2 test is based is :

$$\chi^2 = \frac{(b-4ac)^2\ V}{(2a+b)^2\ \ (b+2c)^2}$$

where a, b and c represent the number of individuals of type M, MN and N respectively in a group of V individuals. The figures for χ^2 are also given in Table III, and fall within acceptable limits.

Relation to World Distribution

A-B-O System

Hirszfeld and Hirszfeld (1919)[8] were the first to study variations of blood groups from a racial aspect. They employed the racial index or " biochemical index "

$$\frac{\%\ \text{Agglutinogen A}}{\%\ \text{Agglutinogen B}} = \frac{\text{Group Frequency A} + \text{Group Frequency AB}}{\text{Group Frequency B} + \text{Group Frequency AB}}$$

The figures obtained by previous workers in Ceylon, together with the present investigation are given in Table V which also gives the racial index in each case.

This method of classifying races is not however, entirely satisfactory, because, as has been pointed out by Wiener[6], they give an incomplete picture. Thus it considers only the relative frequencies of A and B factors, while the actual frequencies of the four groups may show variations which are not accounted for in the biochemical index.

A better test is based on the use of the gene frequencies which have already been calculated for the results of the present investigation. Wiener uses the method of triangular coordinates to represent graphically the serological composition of some of the peoples studied. This is reproduced by kind permission of the publishers (fig. 1)* and the serological composition of the Ceylonese as shown by the present investigation is included in it.

M-N System

Wiener[6] discusses the work done on the racial distribution of the agglutinogens M and N. He cites Mustakallio's method for graphically representing the serological composition of the population (fig. 2).* The gene frequencies m and n are read off on the straight line

* For Figurss 1 & 2, see end of article.

TABLE V

Observed phenotype frequencies of Blood Groups in various samples

Blood Group		*Sinhalese*					*Tamils*					*Total (Including 'Others')*				
		Hill	*Sene-viratne*	*Koch*	*Pre-sent*	*Com-bined*	*Hill*	*Sene-viratne*	*Koch*	*Pre-sent*	*Com-bined*	*Hill*	*Sene-viratne*	*Koch*	*Pre-sent*	*Com-bined*
O	%	47.05	46.42	49.41	47.14	46.80	50.74	45.99	44.22	44.30	45.87	47.87	46.19	48.32	46.39	46.58
	No.	335	1674	168	436	2613	69	258	65	136	528	449	2164	256	604	3461
A	%	26.26	22.82	22.35	23.35	23.32	13.97	18.36	21.77	21.50	19.11	24.52	22.88	22.18	22.89	23.04
	No.	187	823	76	216	1302	19	103	32	66	220	230	1072	117	298	1712
B	%	24.72	26.51	25.88	23.89	25.81	30.88	29.41	27.89	28.34	29.11	25.16	26.28	26.14	25.12	25.92
	No.	176	956	88	221	1441	42	165	41	87	335	236	1231	133	327	1926
AB	%	1.97	4.25	2.36	5.62	4.07	4.41	6.24	6.12	5.86	5.91	2.45	4.65	3.36	5.60	4.45
	No.	14	153	8	52	227	6	35	9	18	68	23	218	18	73	331
Total		712	3606	340	925	5583	136	561	147	307	1151	938	4685	524	1302	7430
Racial Index		1.06	0.88	0.88	0.98	0.92	0.52	0.69	0.82	0.80	0.71	0.98	0.89	0.87	0.93	0.91

and the frequencies of the M and N types of blood groups on the parabola. The gene frequencies are given by the formulae :

$$m = M + \frac{MN}{2} = 62.44$$

$$n = N + \frac{MN}{2} = 37.55$$

This figure is reproduced by kind permission of the publishers with the addition of the frequencies among the combined figures for the Ceylonese. No comment is made on the anthropological deductions to be drawn from these figures, as that would be outside the scope of the present paper.

Exclusion of Paternity

Wiener *et al* (1930)[9] worked out formulae for assessing a falsely accused person's chances of proving non-paternity. These were worked out for the different blood groups that the father might belong to. However, the blood groups of the mother were not considered. Taking these into account considerably extends the variation of chances for, as we shall see, whereas a father of group A has a 11.4% chance of exclusion on the A-B-O system when the blood group of the mother is unknown, he has a 16.5% chance if the mother is in group O or A, and no chance if the mother happens to be in group B or AB.

Calculations are therefore made to estimate the chances of exclusion when the blood groups of the parents are known. These are based on the frequencies of mother-child combinations. Formulae for these have been worked out by Wiener[6] (pages 183 and 241) on the method suggested by Schiff. These formulae and the calculated frequencies are given in Tables VI and VII.

TABLE VI

Frequency of Mother-Child combinations in the total population

(ABO grouping)

Mother's Group	*Children's Group*				
	O	*A*	*B*	*AB*	*Total*
O	r^3 .3160	pr^2 .0702	qr^2 .0763	— —	.4625
A	pr^2 .0702	$p(p^2+3pr+r^2)$ .1204	pqr .0170	$pq(p+r)$ .0207	.2283
B	qr^2 .0763	pqr .0170	$q(q+3qr+r^2)$ .1361	$pq(q+r)$ .0211	.2505
AB	— —	$pq(p+r)$ .0207	$pq(q+r)$ .0211	$pq(p+q)$ .0079	.0497
Total	.4625	.2283	.2505	.0497	1.000

TABLE VII

Frequency of Mother-Child combinations in the total population

(*MN Grouping*)

Mother's Group	*Children's Group*			
	M	*MN*	*N*	*Total*
M	m^3 .2457	m^2n .1466	—	.3923
MN	m^2n .1466	mn .2341	mn^2 .0875	.4682
N	—	mn^2 .0875	n^3 .0522	.1397
Total	.3923	.4682	.1397	1.0002

From these figures it is a simple step to work out the probable frequencies for children of mothers in a particular group. These results are given in tables VIII and IX.

TABLE VIII

Frequencies of Children in Mothers of Different A-B-O Groups

Mother's Group	*Children's Group*			
	O	A	B	AB
O	0.6832	0.1518	0.1650	—
A	0.3075	0.5274	0.0745	0.0907
B	0.3046	0.0679	0.5433	0.0842
AB	—	0.4165	0.4245	0.1590

TABLE IX

Frequencies of Children in Mothers of Different M-N Groups

Mother's Group	*Children's Group*		
	M	MN	N
M	0.6263	0.3737	—
MN	0.3131	0.5000	0.1869
N	—	0.6263	0.3737

Table X and XI give the children that are impossible from any particular parental combination.

TABLE X

Groups impossible in children of different parental combinations
A-B-O System

Mother's Group	*Father's Group* O	A	B	AB
O	A, B, AB	B, AB	A, AB	O, AB (Nil)
A	B, AB	B, AB	Nil	O (Nil)
B	A, AB	Nil	A, AB	O (Nil)
AB	O, AB (Nil)	O (Nil)	O (Nil)	O (Nil)

TABLE XI

Groups impossible in children of different parental combinations
M-N System

Mother's Group	*Father's Group* M	MN	N
M	MN, N	N (Nil)	M, N (Nil)
MN	N (Nil)	Nil	M (Nil)
N	M, N (Nil)	M (Nil)	M, MN

Some of these are impossible only if the second law in each system is accepted, *i.e.*, in the A-B-O system, a group AB parent cannot have a group O child and a group O parent cannot have a group AB child. In the M-N system, a group M parent cannot have a group N child and a group N parent cannot have a group M child. The groups that would be impossible if those laws are not accepted are included in brackets.

Until quite recently these laws were accepted with a certain amount of reserve. Andresen (1952)[10] however, discusses the mass of groupings carried out and comes to the conclusion that exclusion by them "is as accurate as at all possible by a biological method. The possibility of mistaken exclusion is so remote that it can be disregarded". Nevertheless, in the tables worked out, the chances of exclusion that apply if the second set of laws are not accepted, are also included and are enclosed within brackets.

By using the last two sets of tables given above it is possible to calculate the chances of exclusion for any parental combination. When the group of one of the parents is unknown, it is necessary to calculate the average probabilities considering all the possible groups the

unknown parent may belong to. For instance, suppose the mother belongs to group O, and the father's group is unknown. Then, from Table XII it is found that if the father's group was O the probability of exclusion would be .3168, if A it would be .1650, if B .1518 and if AB .6832.

The probability of exclusion in each group should now be multiplied by the frequency of that group in the general population, and the sum of these four products gives the overall probability if the father's group is unknown.

$$(.3168 \times .4625) + (.1650 \times .2283) + (.1518 \times .2505) + (.6832 \times .0497) = .2562.$$

In this fashion it is possible to calculate the probability when the mother's group too is unknown.

Table XII gives the chances using the ABO system.

TABLE XII

Probability of exclusion by ABO system

Mother's Group	*Father's Group* O	A	B	AB	Unknown
O	0.3168	0.1650	0.1518	0.6832 (0)	0.2562 (0.2223)
A	0.1652	0.1652	0	0.3075 (0)	0.1293 (0.1140)
B	0.1518	0	0.1518	0.3046 (0)	0.1233 (0.1082)
AB	0.1590 (0)	0	0	0	0.0735 (0)
Unknown	0.2302 (0.2223)	0.1140	0.1082	0.4625 (0)	0.1826 (0.1559)

Table XIII gives the chances using the MN system.

TABLE XIII

Probability of exclusion by M-N system

Mother's Group	*Father's Group* M	MN	N	Unknown
M	0.3737	0	0.6263 (0)	0.2341 0.1466
MN	0.1869 (0)	0	0.3131 (0)	0.1170 (0)
N	0.3737 (0)	0	0.6263	0.2341 (0.0875)
Unknown	0.2863 (0.1466)	0	0.4788 (0.0875)	0.1793 (0.0687)

Table XIV gives the chances using both systems.

TABLE XIV

Percentage chances of exclusion of Paternity for every possible combination of parents using both A-B-O and M-N systems

Mother's Group		*Putative Father's Group* O				A				B				AB				Unknown			
		M	MN	N	Unknown	M	MN	N	Unknown	M	MN	N	Unknown	M	MN	N	Unknown	M	MN	N	Unknown
O	M	57	32	74 (32)	48 (42)	48	17	69 (17)	36 (29)	47	15	32 (15)	35 (28)	80 (37)	68 (Nil)	88 (Nil)	76 (15)	53 (51)	26 (22)	72 (22)	43 (34)
	MN	44 (32)	32	53 (32)	40 (32)	32 (17)	17	43 (17)	26 (17)	31 (15)	15	42 (15)	25 (15)	74 (Nil)	68 (Nil)	78 (Nil)	72 (Nil)	40 (22)	26 (22)	49 (22)	34 (22)
	N	57 (32)	32	74	48 (38)	48 (17)	17	69	36 (24)	47 (15)	15	32 (68)	35 (23)	80 (Nil)	68 (Nil)	80 (63)	76 (9)	53 (22)	26 (22)	72 (71)	43 (29)
	Unknown	51 (42)	32	64 (38)	44 (36)	40 (29)	17	57 (24)	31 (22)	39 (28)	15	56 (23)	30 (21)	77 (15)	68 (Nil)	84 (9)	74 (7)	47 (34)	26 (22)	61 (29)	39 (28)
A	M	48	17	69 (17)	36 (29)	48	17	69 (17)	36 (29)	37	Nil	63 (Nil)	23 (15)	57 (37)	31 (Nil)	74 (Nil)	47 (15)	4 (44)	13 (11)	33 (11)	67 (24)
	MN	32 (17)	17	43 (17)	26 (17)	32 (17)	17	43 (17)	26 (17)	19 (Nil)	Nil	31 (Nil)	12 (Nil)	44 (Nil)	31 (Nil)	52 (Nil)	39 (Nil)	29 (11)	13 (11)	40 (11)	23 (11)
	N	48 (17)	17	69	36 (24)	48 (17)	17	69	36 (24)	37 (Nil)	Nil	63	23 (9)	57 (Nil)	31 (Nil)	74 (63)	47 (9)	45 (11)	13 (11)	33 (67)	67 (19)
	Unknown	40 (29)	17	57 (24)	31 (22)	40 (29)	17	57 (24)	31 (22)	29 (15)	Nil	48 (9)	18 (7)	51 (15)	31 (Nil)	64 (9)	43 (7)	38 (24)	13 (11)	55 (19)	29 (18)
B	M	47	15	32 (15)	35 (28)	37	Nil	63 (Nil)	23 (15)	47	15	32 (15)	35 (28)	56 (37)	30 (Nil)	74 (Nil)	47 (15)	45 (44)	12 (11)	67 (11)	33 (24)
	MN	31 (15)	15	42 (15)	25 (15)	19 (Nil)	Nil	31 (Nil)	12 (Nil)	31 (15)	15	42 (15)	25 (15)	43 (Nil)	30 (Nil)	52 (Nil)	39 (Nil)	29 (11)	12 (11)	40 (11)	23 (11)
	N	47 (15)	15	32 (68)	35 (23)	37 (Nil)	Nil	63	23 (9)	47 (15)	15	32 (68)	35 (23)	56 (Nil)	30 (Nil)	74 (63)	47 (9)	45 (11)	12 (11)	67 (67)	33 (19)
	Unknown	39 (28)	15	56 (23)	30 (21)	29 (15)	Nil	48 (9)	18 (7)	39 (28)	15	56 (23)	30 (21)	50 (15)	30 (Nil)	64 (9)	43 (7)	37 (24)	12 (11)	54 (19)	28 (17)
AB	M	47 (37)	16 (Nil)	69 (Nil)	36 (15)	37	Nil	63 (Nil)	23 (15)	37	Nil	63 (Nil)	23 (15)	37	Nil	63 (Nil)	23 (15)	42 (37)	7 (Nil)	65 (Nil)	29 (15)
	MN	32 (Nil)	16 (Nil)	42 (Nil)	26 (Nil)	19 (Nil)	Nil	31 (Nil)	12 (Nil)	19 (Nil)	Nil	31 (Nil)	12 (Nil)	19 (Nil)	Nil	31 (Nil)	12 (Nil)	25 (Nil)	7 (Nil)	36 (Nil)	18 (Nil)
	N	47 (Nil)	16 (Nil)	69 (63)	36 (9)	37 (Nil)	Nil	63	23 (9)	37 (Nil)	Nil	63	23 (9)	37 (Nil)	Nil	63	23 (9)	42 (Nil)	7 (Nil)	65 (63)	29 (9)
	Unknown	40 (15)	16 (Nil)	56 (9)	31 (7)	29 (15)	Nil	48 (9)	18 (7)	29 (15)	Nil	48 (9)	18 (7)	29 (15)	Nil	48 (9)	18 (7)	34 (15)	7 (Nil)	52 (9)	24 (7)
Unknown	M	52 (51)	23 (22)	71 (22)	41 (34)	45	11	67 (11)	32 (24)	44 (44)	11	67 (11)	32 (24)	66 (37)	46 (Nil)	80 (Nil)	59 (15)	49 (47)	18 (16)	69 (16)	37 (28)
	MN	37 (22)	23 (22)	47 (22)	32 (22)	28 (11)	11	39 (11)	22 (11)	27 (11)	11	39 (11)	21 (11)	56 (Nil)	46 (Nil)	63 (Nil)	53 (Nil)	34 (16)	18 (16)	44 (16)	28 (16)
	N	52 (22)	23 (22)	71 (70)	41 (29)	45 (11)	11	67	32 (19)	44 (11)	11	67	32 (19)	66 (Nil)	46 (Nil)	80 (63)	59 (9)	49 (16)	18 (16)	52 (68)	24 (23)
	Unknown	45	23	60	37	37	11	54	27	36	11	54	27	62	46	72	56	42	18	57	33

Summary

The A-B-O and M-N groupings of 1302 Ceylonese are given.

The figures are statistically analysed for probability on the basis of accepted theories of inheritance. The work of previous authors in Ceylon is reviewed, and the figures are presented in relation to world distribution.

The chances of exclusion of paternity for a man falsely accused are worked out from first principles, based on the figures in this country.

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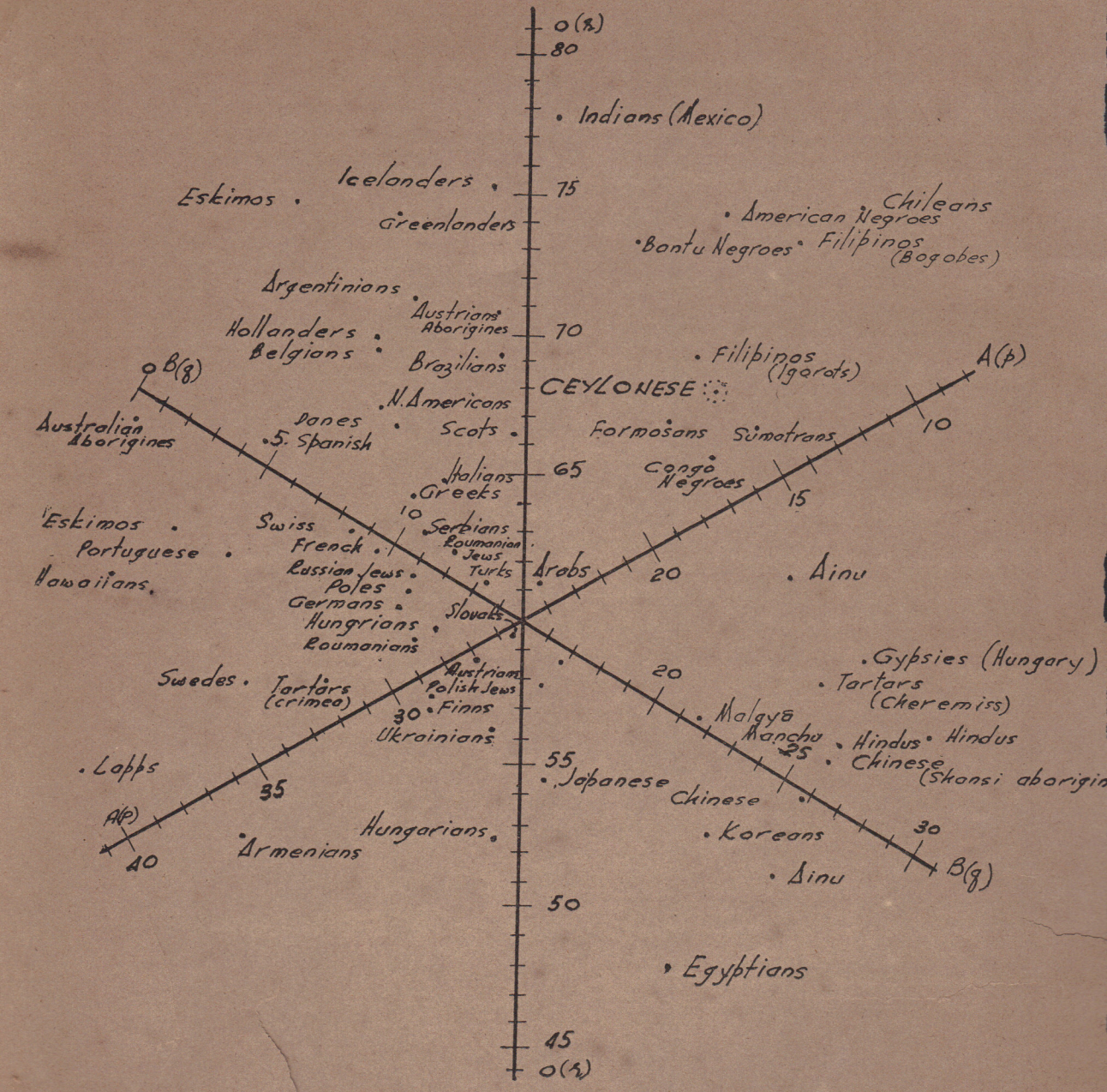

FIG. 1

Serological Composition of Ceylonese in relation to some other peoples throughout the world - A-B-O System
Diagram adapted from "Blood Groups and Transfusion" by Wiener
Third Edition (by courtesy of Charles C. Thomas.

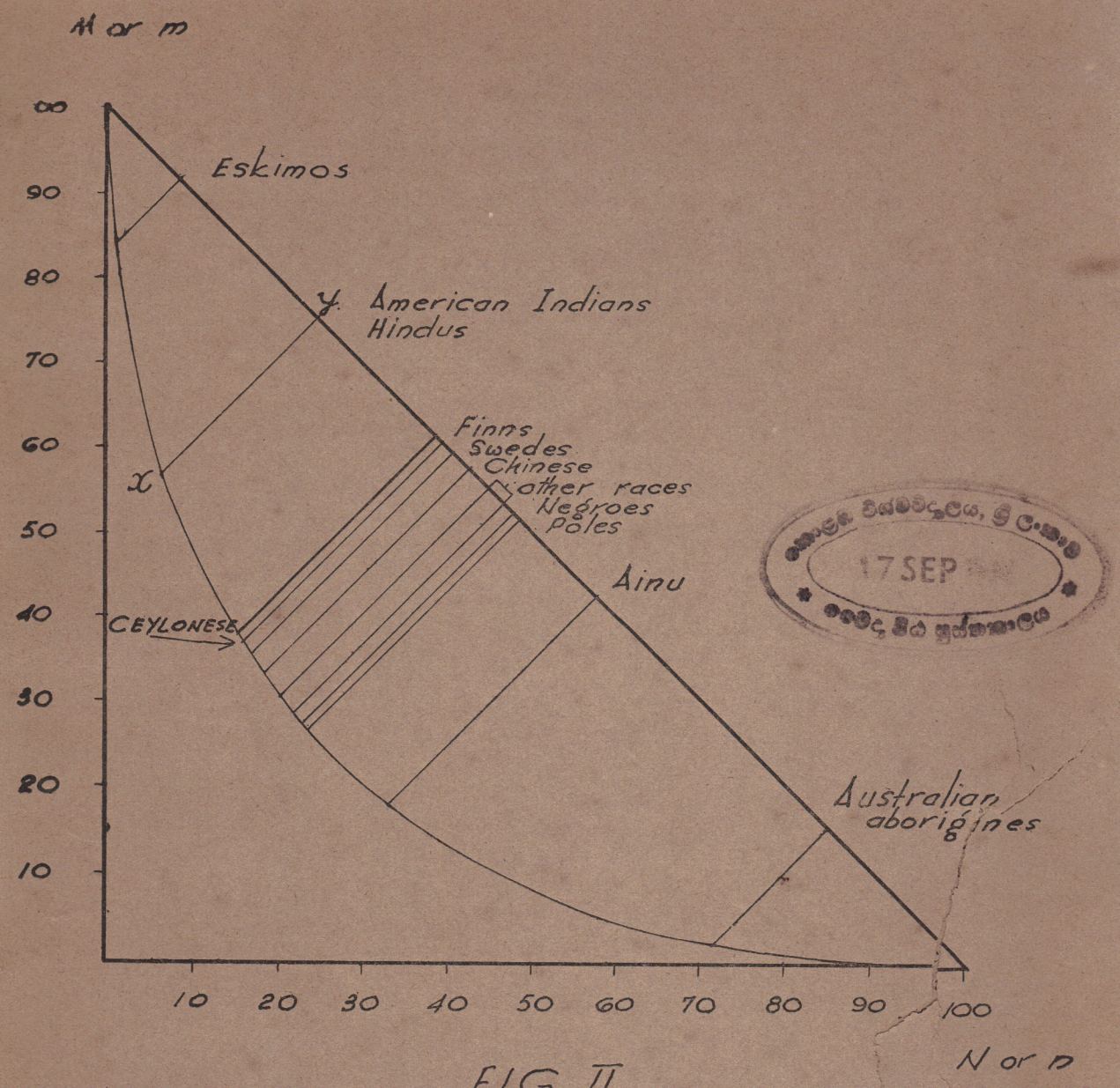

FIG II

Distribution of M and N among Ceylonese in relation to some other peoples.
Diagram adapted from "Blood Groups and Transfusion" by Wiener.
Third Edition (by courtesy of Charles C. Thomas. Publisher Illinois)