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ON THE CONTENT OF GROUP ANTIGEN IN HUMAN SALIVA

BY

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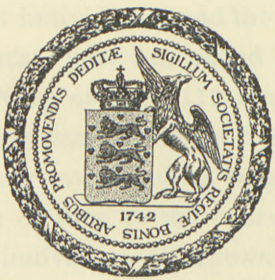

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1. Introduction.

It is a wellknown fact that within a group of individuals distinction may be made between the so-called secreters and non-secreters. The former individuals contain, in their various secretions, group antigen according to their respective blood group, the latter have no group antigen in any of their secretions. (PUTKONEN, SCHIFF and SASAKI, LEHRS and others). This phenomenon was thoroughly studied by SCHIFF and collaborators who found that the "secreter non-secreter phenomenon" had a rather simple heredity, the development of group antigens in the secretions depending upon the presence of a single dominant gene. SCHIFF's experimental material comprised 68 families with 215 children, (total, 351 individuals) and the secretion examined was saliva. MORZYCKI, later on, added 44 families, (total, 202 individuals). None of these 112 families exhibited any exceptions to the genetical theory set forth by SCHIFF. Now, both earlier and later investigations suggested great fluctuations in the course of time in the concentration of antigen in saliva from an individual. (PUTKONEN, LEHRS, HOLZER, DAHR, KAUERTZ). The fluctuation would indeed be so large as to render impossible the diagnosis "secreter" or "non-secreter" on the basis of a single sample of saliva. In spite of such difficulties no serious objections could be raised

against Schiff's genetical hypothesis. On the other hand, definite limits for the antigen concentrations in the two types would seem most desirable with a view to the application of the phenomenon as an aid in forensic medicine. So the elucidation of this point was chosen as the aim of the present research work. The secretion examined was the same as used by Schiff, saliva. The investigations were confined to groups A_1 and B.

2. The Method used for the Determination of the Antigen Concentration.

For the examination of the saliva the agglutination-inhibition method was employed in the following form: Two series of small test tubes were used in the determination of the antigen content of each sample. Into the first tube of each series 0.1 cc of the original antigen solution was introduced, (concentration 1), into the second 0.1 cc of a concentration $^1/_2$, into the third 0.1 cc of a concentration $^1/_4 = \frac{1}{2^2}$, into the fourth 0.1 cc of a concentration $^1/_8 = \frac{1}{2^3}$, etc. Thus into tube no. n 0.1 cc of an antigen solution of concentration $\frac{1}{2^{n-1}}$ was inserted. Hereafter 0.1 cc of a serum anti-A was introduced into all the tubes of one of the two series, while 0.1 cc of a serum anti-B was added to the contents of all the tubes of the other series. Thus, after this operation, the antigen concentrations in the tubes of each of the two series were $\frac{1}{2^1}, \frac{1}{2^2}, \frac{1}{2^3}, \ldots\ldots \frac{1}{2^n}$. In the following experiments the same isosera (anti-A = Ulla $^1/_{14}$—$^1/_{16}$, anti-B = Claus $^1/_{16}$) were employed throughout all the investigations. These two sera proved rather constant as to

strength. After the introduction of serum the test tubes were kept at about 20° C. for an hour when washed blood corpuscles of group A_1 and B were added, the A_1 blood corpuscles to the test tubes with serum iso-anti-A, the B blood corpuscles to the test tubes with serum iso-anti-B. After two hours the tubes were shaken. In one of the two series agglutination was seen to take place in all the tubes, while in the other agglutination only occurred in the tubes following a certain number n (provided that saliva of group A_1 and B only were considered). The number n was taken as a measure of the antigen concentration in the original antigen solution (the saliva) and was termed the titer reading for this solution. It may be noted that n is the power of two in the expression for the antigen concentration in tube no. n after the addition of serum.

If we consider a large number of saliva samples from different subjects and want to know the range within which a certain quality, characteristic of these samples, varies, i. e. what is here termed the indefiniteness of this quality, it is first of all necessary to obtain an idea of the uncertainty of the method used for the measurement of the quality in question. In order to achieve this knowledge 10 titer readings with the same saliva, the same serum and the same test blood corpuscles were made. The observations were further taken on the same day. So the fluctuations in the results must be due solely to the uncertainty of the method. As a measure of this uncertainty the standard deviation μ_u derived from 10 observations was chosen. Below three instances of such a test are given. The standard deviation ranges from about half a titer to one titer. So the method is judged to be fairly satisfactory and suited for the quantitative investigations here dealt with.

Table I.

1) Sample $^{18}/_{9}$ 1939. Saliva A. L. Group A_1. 10 Observations.
Titers 9 12 11 10 10 9 9 9 9 9. Average 9.7.
Standard Deviation $\mu = 1.06$ (or 1.1).

2) Sample $^{25}/_{9}$ 1939. Saliva A. L. Group A_1. 10 Observations.
Titers 9 9 9 9 9 10 9 9 9 9. Average 9.1.
Standard Deviation $\mu = 0.32$ (or 0.3).

3) Sample $^{14}/_{6}$ 1940. Saliva A. L. Group A_1. 10 Observations.
Titers 12 12 13 13 12 13 13 12 13 13. Average 12.6.
Standard Deviation $\mu = 0.52$ (or 0.5).

3. Fluctuations in the Course of Time of the Antigen Concentration in Saliva from a Subject of the Secreter Type chosen at Random (Time to Time Indefiniteness).

From the same person (A. L. group A_1) 39 saliva samples were collected in the course of two months in order to find out what fluctuations of the antigen concentration took place during this time. The samples were generally taken between breakfast and lunch. As will be seen from fig. 1, the fluctuations do not suggest any regular variation in the course of time.

Seeing, however, that these samples of saliva were all collected practically at the same time of the day, it was found appropriate also to consider the fluctuations in the course of a single day in a few subjects. Two persons (V. F. and A. S-H.) of group A_1 were chosen. The results are stated in Tab. II a—b. It is seen that the fluctuations in the subject V. F. are as large as those found in the course of 2 months in the subject A. L. In the case of the former subject a tendency to a higher antigen concentration suggests itself during the hours following the meals. In the individual A. S-H., however, no such correlation could be

Table II a—b.

Fluctuations

in the Antigen Concentration of Saliva from a) a Male (V. F.) of Group A_1 in the Course of two Days, and b) a Female (A. S-H) of Group A_1 in the Course of a Day.

a.

Date	Hour	Titer *n*	V. F's note about his physical condition
Oct. 31 1940	10 a. m.	11	Half an hour after breakfast. Slight depression after a statistical lecture on the previous evening.
	11 a. m.	10	0
	12	11	0
	2 p. m.	12	An hour after lunch.
	3 p. m.	12	10 minutes after eating candy.
	4 p. m.	10	0
	5 p. m.	10	Drinking tea. Just after eating 3 honeycakes.
Nov. 1 1940	12	8	Just after a lecture for the students. Minute secretion of saliva.
	1 p. m.	10	A quarter of an hour after lunch. Drinking coffee.
	3 p. m.	11	10 minutes after having finished a lecture.
	4 p. m.	13	Just after coffee.
	5^{30} p. m.	8	0
	7^{45} p. m.	11	A quarter of an hour after dinner.
	9^{45} p. m.	13	0
	11 p. m.	13	0

Table II a—b cont.

Date	Hour	Titer *n*	V. F's note about his physical condition
Nov. 2 1940	8 a. m.	9	Just awoken.
	9^{30} a. m.	10	Immediately after breakfast and after rinsing the mouth.
	11 a. m.	10	Eating liquorice. (The saliva sample of a dark brown colour).
	12	9	0
			All the saliva samples coloured by tobacco.

b.

Date	Hour	Titer *n*	A. S-H's note about her physical condition
Nov. 6 1940	11 a. m.	8	0
	12	8	0
	1 p. m.	9	Dinner 12^{30} + coffee + a cigarette.
	2 p. m.	8	0
	3 p. m.	9	0
	4 p. m.	8	Drinking coffee.
	5 p. m.	9	Smoking a cigarette.
	6 p. m.	9	0
	7 p. m.	8	A quarter of an hour after evening meal.
	8 p. m.	9	0
	9^{20} p. m.	10	0
	10^{20} p. m.	9	A quarter of an hour after coffee + a cigarette.
	11 p. m.	8	0
	12	9	0

Table II a—b cont.

Date	Hour	Titer *n*	A. S-H's note about her physical condition
Nov. 7 1940	4 a. m.	8	0
	8^{30} a. m.	10	Drinking coffee.
	9^{30} a. m.	10	0
	11 a. m.	9	0
	12	10	0

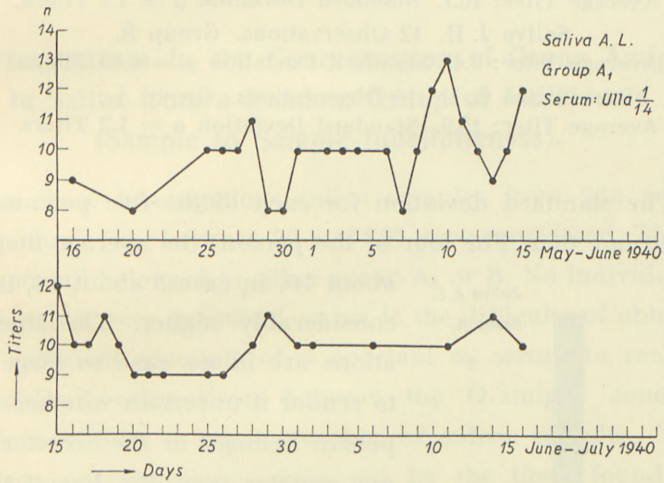


Fig. 1. Fluctuations in the Course of two Months in the Concentration of the Group Antigen in Saliva from a Person of Group A_1.

traced and so the tendency referred to is very likely accidental or, most probably, it is inessential at what hour of the day the samples are collected.

Again, salivas from five persons were examined at different times during a period of one to three years. Fig. 2 represents the distribution of the titer readings for saliva obtained from one of the five individuals in the course of three years.

The distribution curve is rather regular, showing no greater fluctuations than the saliva samples in fig. 1. Tab. III shows the average titers and standard deviations for all five persons

Table III.

1) Saliva V. F. 25 Observations. Group A_1.
Average Titer: 10.0. Standard Deviation $\mu = 0.89$ Titers.

2) Saliva A. L. 57 Observations. Group A_1.
Average Titer: 9.9. Standard Deviation $\mu = 0.96$ Titers.

3) Saliva E. R. 14 Observations. Group B.
Average Titer: 10.1. Standard Deviation $\mu = 1.1$ Titers.

4) Saliva J. H. 12 Observations. Group B.
Average Titer: 9.4. Standard Deviation $\mu = 0.89$ Titers.

5) Saliva G. M. 16 Observations. Group A_1.
Average Titer: 13.9. Standard Deviation $\mu = 1.2$ Titers.

The standard deviation for each of the five persons is about one titer. For four of the persons the average titer is about 10, in case 5 about 14, thus considerably higher. The fluctuations are in no case so large as to render it uncertain whether the person belongs to the secreter or non-secreter type, the lowest titer reading being as high as titer 7. Now, the standard deviations μ_A found in the five cases cannot be taken as a true expression of the fluctuations in the course of time (the time to time indefiniteness) of the antigen concentration in saliva from a subject chosen at random, seeing that these determinations also include the indefiniteness due

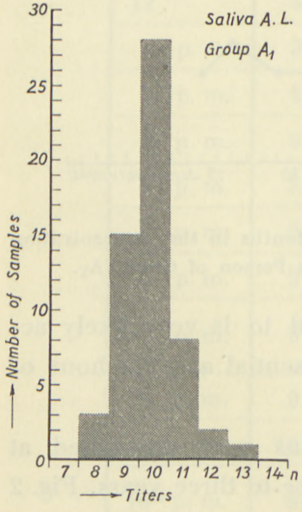


Fig. 2. Distribution of the Antigen Concentrations within 57 Saliva Samples from the same Individual.

to the uncertainty of the method, μ_U. This indefiniteness was found to be $\mu_U = 0.63$ (average value, compare Tab. I), while μ_A was found to have an average value of 1.01. From the expression

$$\mu_A^2 = \mu_T^2 + \mu_U^2$$

the actual time to time indefiniteness μ_T is determined:

$$\mu_T = \sqrt{1.01^2 - 0.63^2} = 0.79 \text{ titers.}$$

4. Fluctuations in the Concentration of Group Antigen in Saliva from a Random Group of Individuals (Sample to Sample Indefiniteness).

In these investigations saliva samples from 263 adult individuals (age between 20 and 50) were considered. These persons all belonged to either group A_1 or B. No individuals of group 0 were examined owing to the difficulty of obtaining sufficient amounts of a constant α_2 serum to render possible a comparison between the O-antigen concentrations within a larger number of saliva samples. The antigen concentrations, measured by the titers found in agglutination-inhibition tests, are stated in Tab. IV while in fig. 3 the experimental material is represented in a block diagram.

First of all it will be noted that there is a distinct interval, covering the titers 4, 5, and 6, between the secreter- and non-secreter groups. So the assumption maintained by earlier authors of a continuous transition between the two groups must be discarded. ("Vollausscheider, Teilausscheider, Nichtausscheider"). Again it is seen that the non-secreter salivas are not, all of them, quite devoid of group

Table IV.

Distribution of the Concentrations of the Group Antigen within a Random Group of 263 Adults belonging to Groups A_1 and B.

Titer *n*	Number of Saliva Samples	Percentage Number of Saliva Samples
0	57	21.7
1	7	2.7
2	3	1.1
3	1	0.4
4	0	..
5	0	..
6	0	..
7	1	0.4
8	4	1.5
9	26	9.8
10	29	11.0
11	30	11.4
12	16	6.1
13	18	6.8
14	23	8.7
15	20	7.6
16	14	5.3
17	7	2.7
18	5	1.9
19	2	0.8

antigen. The latter fact, too, is also at variance with statements by earlier authors. Now, most likely these authors have employed titer scales with a zero-point different from that of our scale, say a zero-point at the titer 8 in this scale.

This would mean 1) that secreters sometimes would be diagnosticated as "non-secreters" and 2) that genuine non-secreters never would be found to contain any trace of antigen.—It must of course be realised that the zero-point of our scale is also accidental, seeing that the titer 0 stands for all antigen concentrations below titer 1.

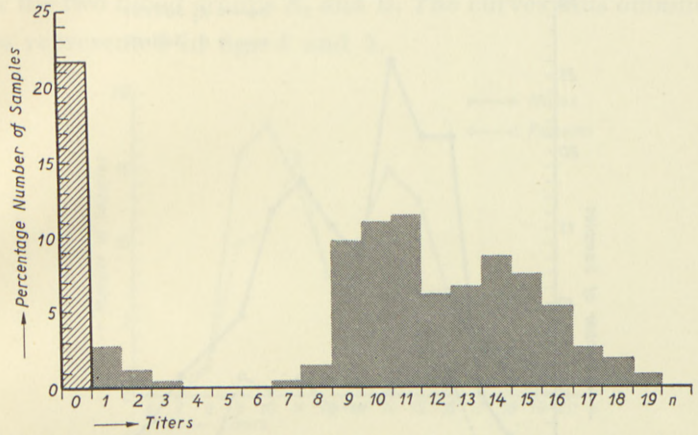


Fig. 3. Distribution Diagram for the Concentrations of Group Antigen in Salivas from 263 Adults of Groups A_1 and B.

From Tab. III and fig. 3 it is further seen that the secreter group comprises 74 % and the non-secreter group 26 %— in good agreement with the figures found by PUTKONEN and by SCHIFF. The standard deviation for the secreter group is $\mu'_A = 2.7$ titers. This, however, is not the actual fluctuation in antigen concentration in saliva from a random group of individuals or what may be termed "the sample to sample indefiniteness" μ_S, seeing that μ'_A also includes the indefinitenesses due to the uncertainty of the method μ_U and the "time to time indefiniteness" μ_T for an individual chosen at random. These two indefinitenesses were 0.63 and 0.79 titers respectively. So the actual sample to sample indefiniteness is determined from

$$\mu_S^2 = \mu_{A'}^2 - \mu_U^2 - \mu_T^2 = 2.7^2 - 0.63^2 - 0.79^2$$

or $\mu_S = 2.5$ titers.

Thus the apparent indefiniteness 2.7 titers is not very far from the actual sample to sample indefiniteness. Another feature of the block diagram fig. 3 is as follows. It would

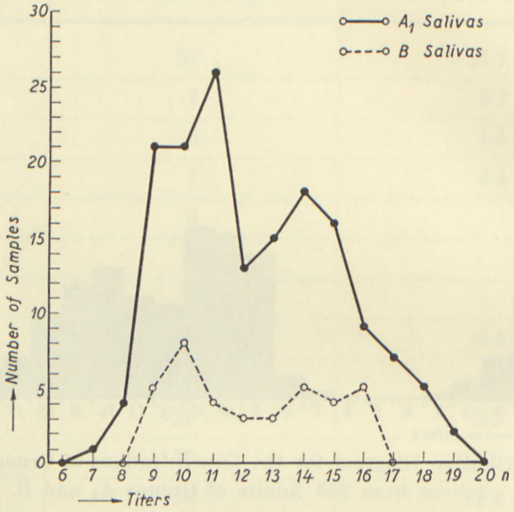


Fig. 4. Distribution Curves for the Concentrations of Group Antigens in Saliva from Secreters of Groups A_1 and B.

seem that two maxima, one at the titers 10 to 11 and the other at titer 14, may be traced within the secreter group of this diagram. As will be remembered from the investigations on the fluctuations in the course of time of the antigen concentration in saliva from a subject chosen at random, four of these showed an average concentration at about titer 10 while one person had the average concentration at titer 14. Thus these two values coincide with the two maxima in the block diagram fig. 3. This, however, may be accidental, and, on the other hand, the two maxima in fig. 3 may reflect the inhomogeneity of the material only.

Here it may be noted that the material comprised saliva both from persons of group A_1 and B and from both men and women.

In order to find out whether the two maxima are due to one of these circumstances, separate curves were drawn on the basis of the secreter material for the two sexes and for the two blood groups A_1 and B. The curves thus obtained are represented in figs. 4 and 5.

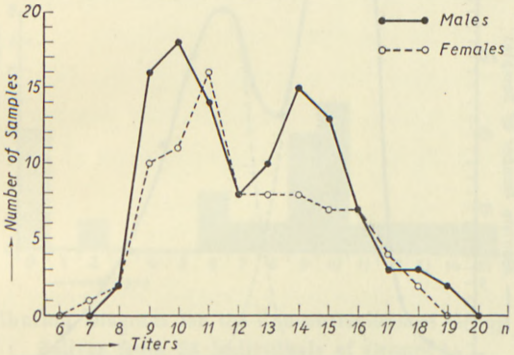


Fig. 5. Distribution Curves for the Concentrations of the Group Antigens A_1 and B in Saliva from Males and Females.

Obviously all four curves exhibit the characteristic division into two groups with different maxima. So it would seem rather safe to state that the secreter curve drawn from the total material is in fact resultant of two overlapping curves. Further the analysis of the material shows that it was quite justifiable not to distinguish between the two blood groups A_1 and B, or between the sexes in our material.

In fig. 6 an attempt is made to separate the two secreter curves. The areas of the two curves are about as 7 to 9, the smallest area being that of the curve covering the higher antigen concentrations. Now, in the explanation of this

twin curve there are two possibilities[1]. The gene on which the presence of antigen in the saliva depends may not be absolutely dominant, resulting in a weaker secreter type in the heterozygote individual. This hypothesis would seem the more probable. On the other hand it cannot be al-

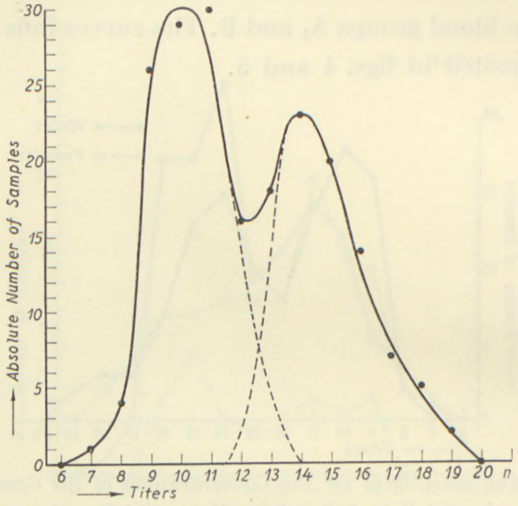


Fig. 6. Distribution Curve for the Antigen Concentrations in Salivas from Secreters of Groups A_1 and B.

together precluded that the twin curve may reflect the presence in the experimental material of two groups of individuals of which one is the heterozygote with regard to the genes determining the blood groups, the other the homozygote. In this connection it should be noted that the material consisted exclusively of persons of the groups A_1 and B. The way to settle this question would be to collect an experimental material either from persons of group 0 or from persons of group A_1 B. The former scheme would

[1] A third possibility, *viz.* the existence of two different secreter genes, could of course be theoretically maintained. This hypothesis is, however, less plausible so it will not be entertained below.

be the more difficult because of the inhomogeneity of the α_2 sera. The latter would of necessity take a rather long time seeing that about 3 % only of all individuals belong to that blood group. So neither of the tests indicated have been carried out.

An attempt was made to investigate whether the quantitative distribution of the individuals within the two secreter groups fits in with the theory of the gene frequencies. The

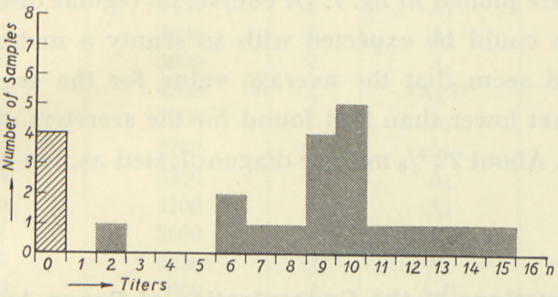


Fig. 7. Distribution Diagram for the Concentrations of Group Antigens in Salivas from 23 Individuals of Group A_2.

assumption was made that the twin curve reflects the presence of heterozygote and homozygote subjects with regard to the secreter gene S. Then the relative frequencies of the various types are found to be (compare figs. 3 and 6):

1) SS = 32 %
2) Ss = 42 % } Secreters
3) ss = 26 % Non-secreters.

Denoting the gene frequency of type SS as α^2, that of type ss as β^2, the frequency of type Ss is $2\alpha\beta$. Introducing α and β, (the square roots of the percentage frequencies found), into the equation

$$\alpha + \beta = 1$$

we find

$$\sqrt{0.32} + \sqrt{0.26} = 0.57 + 0.51 = 1.08$$

in fairly good agreement with the theoretical value 1.00, the uncertainty of the division of the twin curve into two separate curves being considered.

A few saliva samples from persons of group A_2 were examined in connection with the main investigations. The results are plotted in fig. 7. Of course, no regular distribution diagram could be expected with so scanty a material, but it would seem that the average value for the secreters is somewhat lower than that found for the secreters of groups A_1 or B. About 22 % may be diagnosticated as non-secreters.

5. Fluctuations in the Concentration of Group Antigen in Saliva from a Random Group of newborn Infants.

From the Maternity Ward A in the State Hospital of Copenhagen, (Rigshospitalet i København), saliva samples from 120 newborn infants (58 boys and 62 girls) were collected. The blood group characters of the children were in all cases determined from blood obtained from the umbilical cord. All the children were diagnosticated as full-grown and the saliva samples were taken within 4 to 6 days after birth. The material comprised mainly children of groups A_1 and B, but a few infants of the groups A_2 and AB were included. Tab. V contains the total material.

From the latter the individuals of group A_1 and B (and A_1B) were selected and the percentage distribution of the antigen concentrations calculated. The results are

Table V.

Distribution of the Concentrations of Group Antigens in Salivas from 120 newborn Infants.

Number of the Sample	Weight of the Child	Sex	Blood Group	Saliva-Titer *n*
744	2900 g	♂	A_1	8
748	3200	♂	B	8
758	3250	♀	A_1	10
749	2450	♀	A_1	12
747	2000	♀	A_2	4
759	3900	♀	A_1	1
752	4200	♂	$(A_2)B$	7
764	2900	♀	A_1	7
773	2700	♀	A_1	6
774	3300	♂	A_1	5
780	4100	♂	A_1	6
781	3500	♀	A_1	4
783	3350	♂	B	7
762	3600	♂	A_2B	0—2
796	3300	♂	A_1	4
782	2750	♂	A_1	1
787	3150	♀	A_1	6
791	3300	♀	A_2	6
794	3850	♀	A_2	6
793	3100	♂	A_1	7
814	2600	♀	B	4
811	2750	♀	A_1	7
812	3200	♂	A_2	7
806	2900	♂	A_1	6
809	2600	♂	A_1	6
825	2700	♀	A_2	6
824	3300	♀	A_1	8
792	3200	♀	A_1	6
829	3600	♀	A_1	2
826	3600	♀	A_1	7
840	4000	♂	A_1	6
834	3300	♀	A_1	7
837	3650	♀	A_1	6
821	2900	♀	B	6
842	2900	♂	A_1	6
846	3000	♀	A_1	7

Table V cont.

Number of the Sample	Weight of the Child	Sex	Blood Group	Saliva-Titer *n*
820	3400 g	♀	B	5
853	3000	♂	B	11
847	2900	♂	A_1	11
848	2900	♀	A_1	6
863	3300	♀	A_1	10
864	3500	♂	A_1	7
867	3350	♀	A_1	9
865	3600	♂	A_1	8
819	2900	♂	B	8
880	2950	♂	A_1	5
875	3300	♂	A_1	7
876	3000	♀	A_1	5
872	3400	♂	A_1	6
871	2900	♀	A_1	2
885	2900	♂	A_1B	9—9
888	3600	♂	A_1	7
890	2450	♀	A_2	0
906	2500	♀	A_1	6
910	3150	♀	A_1	0
893	3850	♀	A_1	5
922	2750	♀	A_1	7
920	2950	♀	A_1	6
921	3500	♂	A_1	7
919	3750	♀	B	9
934	3400	♀	A_1	7
939	2800	♀	A_1	4
936	3300	♀	B	6
935	4050	♂	A_1	7
947	3300	♀	A_1	3
904	3800	♂	A_1	7
957	3500	♂	(A_3?)B	3—10
951	3900	♀	A_1	7
949	3600	♀	A_1	5
952	3300	♂	A_1	7
953	3650	♀	A_1B	7—8
956	3200	♀	A_1	5
961	3100	♀	A_1	10
958	2500	♂	A_1	7
987	3400	♂	A_1	6

Table V cont.

Number of the Sample	Weight of the Child	Sex	Blood Group	Saliva-Titer *n*
968	3000 g	♂	A_1	3
972	3800	♀	A_1	7
1002	2800	♀	A_1B	6—8
1000	3450	♂	A_1	7
1007	3200	♀	B	3
1013	3350	♀	A_1	7
1022	3850	♀	A_1	7
1019	2700	♂	A_1	5
1027	3600	♂	A_2	1
868	3300	♀	B	11
1020	..	♀	A_1	8
1043	3800	♂	A_1	5
1026	2550	♀	B	3
1041	3000	♀	B	7
1046	3500	♂	A_1	6
1055	3300	♂	A_1	6
1060	2950	♀	A_1	4
1061	4100	♀	A_2	2
1038	3600	♂	B	11
1049	3000	♂	A_1B	0—2
1045	3300	♂	A_1	6
1073	3200	♂	B	5
1071	4100	♂	B	6
1070	2300	♂	B	6
1077	3050	♂	A_1B	7—7
1076	2900	♀	A_1	6
1072	2700	♀	A_1	1
1082	2800	♂	A_1	5
1083	3300	♂	A_2	0
1086	3500	♂	A_1	6
1089	2650	♀	A_1	7
1093	3250	♂	B	9
1097	3300	♀	B	1
1094	3100	♀	A_1	9
1105	4350	♂	A_1	10
1112	3000	♀	A_2	6
1114	3100	♂	A_1	6
1118	3900	♂	A_1	6
1092	3500	♀	B	8

Table V cont.

Number of the Sample	Weight of the Child	Sex	Blood Group	Saliva Titer *n*
1127	3600 g	♀	A_1B	3—2
1137	3600	♂	A_2	5
1075	3800	♂	B	7
1136	3150	♂	A_1	4
1144	2800	♂	A_1B	0—0
1151	3700	♂	A_1	5

Table VI.

Distribution of the Concentrations of Group Antigens in 116 Saliva Samples from a Random Group of newborn Infants of the Groups A_1 and B (and A_1B).

Titer *n*	Number of Saliva Samples	Percentage Number of Saliva Samples
0	4	3.5
1	4	3.5
2	5	4.3
3	5	4.3
4	6	5.2
5	12	10.3
6	26	22.4
7	29	25.0
8	9	7.8
9	6	5.2
10	5	4.3
11	4	3.5
12	1	0.8
13	0	..

entered in Tab. V from which the block diagram fig. 8 is drawn.

A comparison with the diagram fig. 3 reveals a striking difference between the distribution of the antigen concentration in saliva from adults and from newborns in

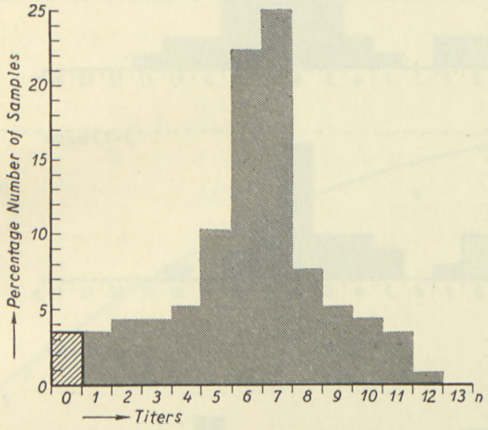
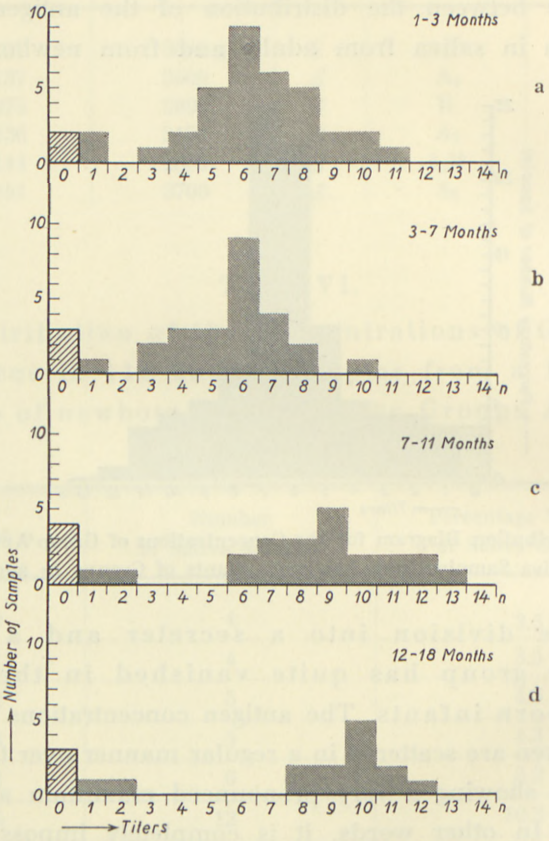


Fig. 8. Distribution Diagram for the Concentrations of Group Antigens in 116 Saliva Samples from Newborn Infants of Groups A_1 and B.

that the division into a secreter and a non-secreter group has quite vanished in the case of newborn infants. The antigen concentrations for all the children are scattered in a regular manner over the first 12 titers, showing a very pronounced maximum at titers 6 to 7[1]. In other words, it is completely impossible to distinguish between secreters and non-secreters in persons so young. On the other hand it follows from this experience that most of the non-secreters to be must contain a certain

[1] According to W. JOHANNSEN a distribution curve with a pointed maximum and great lateral extensions is often characteristic of cases where exterior factors may influence the development of the quality in question. Otherwise the same shape of curve may reflect a mixture of two cases with the same average but with different indefinitenesses.

amount of antigen at birth, while the secreters, must as a rule, exhibit a lower concentration of antigen in the saliva than the adults. One might imagine that the lower titers,



Figs. 9 a—d. Distribution Diagrams showing the Concentrations of Group Antigens in Salivas from a Random Group of Children during the first 18 Months of Life.

say up to titer 4 or 5, represent the non-secreters to be, while the remainder cover those individuals which are later on recognized as secreters. The conditions, however, are not so simple, as will be gathered from experiments which will be recorded below.

From various infant homes samples of saliva were collected from children at ages ranging from 1 to 18 months. All the children belonged to group A_1 and B. The concentrations of antigen were determined for the samples and the whole material divided into groups comprising a) the children between 1 and 3 months, b) those between 3 and

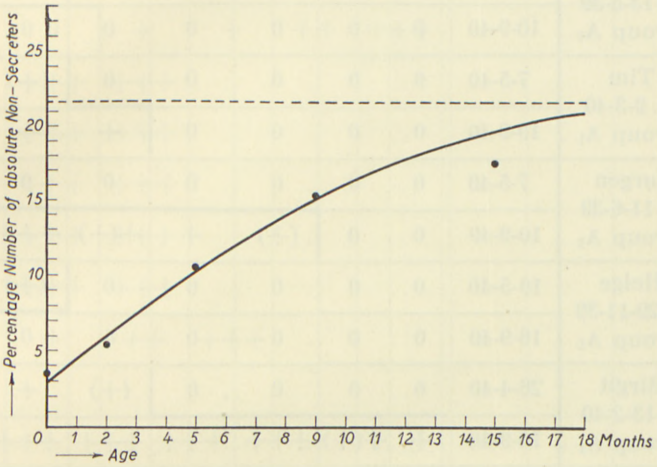


Fig. 10. Graph showing the Percentage Number of Non-Secreters exhibiting the Titer 0, at various Times during the first 18 Months of Life. The dotted Line above represents the average Number of absolute Non-Secreters in Adults.

7 months, c) the children from 7 to 11 months and d) the remainder ranging in age from one year to one year and a half. The concentrations from the four groups were plotted in block diagrams, figs. 9 a—d.

From these diagrams the conclusion may be drawn that within the first half year of life no absolute distinction can be made between secreters and non-secreters, the conditions being chiefly the same as were found for newborn children, (compare fig. 8). After this period a rapid development would seem to take place resulting in a clear cut separation

Table

Repeated Observations on the Concentration of Group

Saliva	Date of Examin.	1	2	3	4	5	6	7	8
Finn b. 13-5-39 Group A_1	7-5-40	0	0	0	0	0	0	0	0
	10-9-40	0	0	0	0	0	0	0	0
Tim b. 9-3-40 Group A_1	7-5-40	0	0	0	0	0	(+)	(+)	+
	10-9-40	0	0	0	0	+	+	+(+)	+++
Jørgen b. 11-6-39 Group A_1	7-5-40	0	0	0	0	0	0	(+)	+
	10-9-40	0	0	(+)	+	+(+)	+++	+++	+++
Helge b. 29-11-39 Group A_1	10-5-40	0	0	0	0	0	(+)	+	+(+)
	16-9-40	0	0	0	0	0	0	0	0
Birgit b. 13-2-40 Group A_1	26-4-40	0	0	0	0	(+)	+	+(+)	+++
	16-9-40	+	+(+)	+++	+++	+++	+++	+++	+++
Kay b. 25-2-40 Group A_1	16-4-40	0	0	0	0	0	0	(+)	+
	16-9-40	0	0	0	0	0	0	0	(+)
Lise Lotte b. 12-5-39 Group A_1	31-12-39	0	0	0	0	0	0	0	0
	22-5-40	0	0	0	0	0	0	0	0
Robert b. 16-9-39 Group B	5-4-40	0	0	0	0	0	(+)	+	+
	18-9-40	0	0	0	0	0	0	0	0
Neel b. 10-10-39 Group A_1	27-3-40	0	0	0	0	0	0	0	(+)
	22-9-40	0	0	0	0	0	0	0	0
Bent b. 10-9-39 Group A_1	20-3-40	0	0	0	0	0	0	(+)	+
	22-9-40	0	0	0	0	0	0	0	0

VII.

Antigen in Saliva from a Number of Individuals.

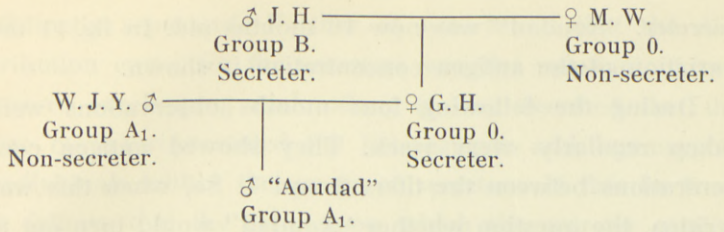
9	10	11	12	13	14	15	16	17	18
0	(+)	+	+(+)	+++	+++	..	..	..	..
0	0	0	+	+	++	+++	..	..	..
+(+)	++	+++	+++	..	..	..	..	..	..
+++	+++	+++	+++	..	..	..	..	..	..
+(+)	++	+++	+++	..	..	..	..	..	..
+++	+++	+++	+++	..	..	..	..	..	..
++	+++	+++	+++	..	..	..	..	..	..
0	(+)	+	++	+++	..	..	..	..	..
+++	+++	..	..	..	..	..	..	..	..
+++	+++	..	..	..	..	..	..	..	..
+(+)	++	+++	+++	..	..	..	..	..	..
+	++	+++	+++	..	..	..	..	..	..
0	0	0	0	0	(+)	+	+(+)	++	+++
0	(+)	+	+	+(+)	++	++	+++	..	..
+(+)	++	+++	+++	..	..	..	..	..	..
0	+	+	++	+++	..	..	..	..	..
+	+(+)	++	+++	..	..	..	..	..	..
0	0	(+)	+	+(+)	+++	..	..	..	..
+(+)	++	+++	+++	..	..	..	..	..	..
0	0	+	+(+)	+++	+++	..	..	..	..

of the two types. Values equal to those found for adults may be reached in the course of the second year. Judging from the rather scanty material of children of the age of 12 to 18 months the titer for non-secreters is perhaps a little higher than in adults, while for the secreters it is perhaps a little lower than in grown-up people. The deviations from the final values are, however, rather small. The results obtained are perhaps represented more clearly by means of a curve, fig. 10.

This curve represents the percentage numbers of absolute non-secreters at various ages; absolute non-secreters meaning subjects who in their saliva show an antigen concentration indicated by the titer 0. The points of the curve are derived from the various distribution diagrams found in this paper. The curve exhibits a regular increase during the first year.

In order to examine more directly the development of the antigen concentration in the saliva two to four observations were taken on a number of the children during the first 18 months of their lives. The examinations, however, failed to give any particularly interesting information. In Tab. VI results from ten children are given.

The case "Jørgen" is a little irregular, showing the titer 6 at an age of 11 months, which titer, judging from the block diagram fig. 9, would indicate that the subject belongs to the secreter group. Four months later titer 2 was found, thus a titer within the non-secreter region. The following report shows that such irregularities are certainly not altogether unique at this early time of life. The author's son "Aoudad", of the group A_1 was regularly examined for the content of group antigen in the saliva during the first 20 months of his life. The pedigree of the child was as follows:



It follows from the scheme that the child has had the same chance of becoming a heterozygote secreter and a

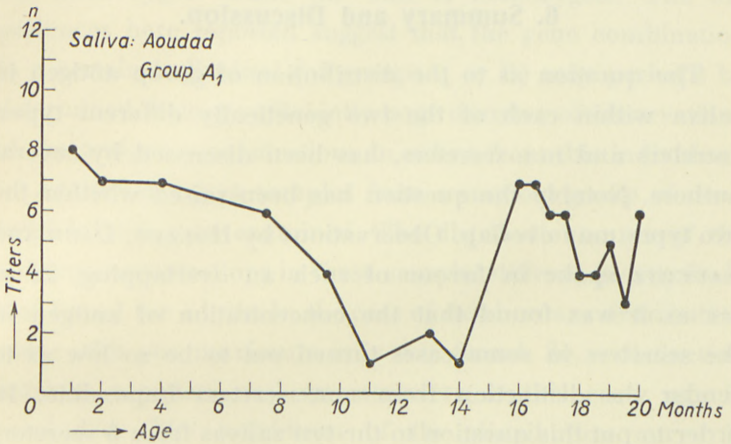


Fig. 11. The Concentration of A-Antigen in the Saliva from a Boy of Group A_1, during the first 20 Months of his Life.

non-secreter. Now, "Aoudad" started at an age of one month, when the observations were initiated, with an antigen concentration of 8 titers. Thus he was deemed to be a secreter. During the next 10 months the antigen concentration, however, smoothly diminished, reaching distinct non-secreter values when the child was 11 months old. These values remained constant for three months. Then after an interruption of the observations during two months of summer holidays a new test disclosed an increased amount of antigen, though not as great as normal in a grown-up

secreter. "Aoudad" was now 16 months old. In fig. 11 the variation of the antigen concentration is shown.

During the following four months observations were taken regularly every week. They showed antigen concentrations between the titers 3 and 7. So, when this was written, the question whether "Aoudad" would turn out to be a secreter or a non-secreter could not be settled.

6. Summary and Discussion.

The question as to the distribution of group antigen in saliva within each of the two genetically different types, secreters and non-secreters, has been discussed by several authors. Notably the question has been raised whether the two types may overlap. Observations by Holzer, Dahr and Kauertz spoke in favour of such an overlapping, in so far as it was found that the concentration of antigen in the secreters in some cases turned out to be so low as to render the distinction from non-secreters impossible. In order to put this question to the test salivas from 5 secreters of group A_1 and B were examined during a period of 3 years. The fluctuations of the antigen concentrations in these 5 individuals were all found to keep within certain rather definite regions clearly separated from the corresponding regions for non-secreters. It should be noted that four of the secreters in question exhibited coinciding distribution curves with an average concentration at about titer 10 while the average for the subject no. 5 was about 14.

Again the antigen concentrations in salivas from 263 adult individuals chosen at random among persons of groups A_1 and B were measured. In this case, too, the distribution curves for the secreters and non-secreters were

distinctly separated. Further, it was found that the distribution curve for the secreters was the resultant of two curves overlapping each other. It was assumed that the two curves represented the heterozygote and homozygote individuals within the group of secreters. If this be true, it follows that the gene upon which the secreter quality depends is not absolutely dominant. Again it may be assumed that four of the five individuals mentioned above are heterozygote while the fifth is homozygote. The experiences here reported suggest that the gene combination in a certain individual (of group A_1 or B) may perhaps be determined from a sufficiently large number of observations of the antigen concentrations in saliva from this individual.

Again 120 saliva samples from a random group of newborn infants were examined. The distribution curve of the antigen concentrations in these samples differed greatly from that found for adults. In the case of newborns, a distribution curve with one maximum only was found. So, a distinction between secreters and non-secreters at this early age is precluded. The maximum of the distribution curve is situated at titer 6 to 7, i. e. in the interspace between the curves for secreters and non-secreters in the case of adults. The differentiation of the two types would seem to take place during the period from 6 months to 18 months judging from observations on a number of children at ages within this interval. It appears, however, that certain rather rare deviations from this rule may occur.

The facts here stated may be thus summarised: It has been established that a hereditary quality, distinctly expressed at birth, may, according to the genotype of the individual, either grow more pronounced or practically disappear in the course of time.—An analogy to this phenom-

enon may perhaps be found in the development of the colour of the iris. As is well known, the colour of the eyes in newborn infants is uniformly of a dark blue shade. During the following months this colour either develops into a decided blue or it loses every trace of blue and assumes various shades of brown depending on the genotype of the individual. Now it should be borne in mind that the group antigens probably have the character of polysaccharides (LANDSTEINER). It may naturally be assumed that these polysaccharides play some necessary part in the organism. If this be true it follows that the non-secreters also must possess these polysaccharides although in a form showing no group specificity. Now, it is known that the manifestation of a certain specificity often depends on relatively minute structural characteristics. So it would not seem precluded that such molecular characteristics might develop even during the growth of the individual. The final development of the non-secreter type might for instance depend on the production of polysaccharides able to give faint cross-reactions with the genuine group specific polysaccharides, or it might reflect the gradual development of two slightly different polysaccharides: a tiny part equal to the genuine group specific polysaccharide and the remainder not exhibiting this specificity.

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(From the Institute of Forensic Medicine,
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Chief: Professor KNUD SAND).

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