106. *On the Subgroups of O Blood Groups.*

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Introduction.

Although it is already known that A blood groups can be divided into two subgroups, A_1 and A_2 , B blood groups²⁾ into B_1 and B_2 , and AB blood groups into A_1B_1 , A_1B_2 , A_2B_1 and A_2B_2 , it has long been believed impossible to divide O blood groups into subgroups.

The study of dividing O blood groups into subgroups began with the research of M. Hibino²⁾ (1935), when he noticed that there was an individual difference in the agglutinability of O blood corpuscles to heteroagglutinin found in the normal sera of animals. Following this research, Maekawa³⁾ (1944) immunized fowls with the kidney cell emulsion of guinea pigs, and prepared anti- O_{II} serum; he then gave the name O_1 to the O blood groups which came to be strongly agglutinated by this antibody, and the name O_2 to the O blood groups which were hardly agglutinated at all. Tokugawa⁴⁾ (1946) immunized fowls with the red blood corpuscles of the white rat, prepared anti- O_{II} serum, and succeeded in dividing O blood groups into two subgroups. Morgan and Watkins⁵⁾ (1948) immunized some animals with the blood corpuscles of cows and rabbits or human O blood groups, and caused this immunesera to act on a number of O blood groups; they gave the name "H substance" to the human O substance that resembled the O substance contained in the blood corpuscles of pigs, and called the other "the real O substance", thus dividing O blood groups into two subgroups. M. Murakami⁶⁾ (1950) tried to divide O blood groups into subgroups and, according to the method reported by Maekawa and Tokugawa, immunized fowls with the kidney cell emulsion of guinea pigs, and thus prepared anti- O_{II} serum. This anti- O_{II} serum agglutinates human blood cells in the following order, i. e., O blood groups, E blood cells and A_2e blood cells.

O_1 and O_2 Subgroups of O Blood Corpuscles.

If we allow the anti- O_{II} serum prepared in the above manner to be absorbed by O blood corpuscles of weaker agglutinability, the supernatant serum will no longer agglutinate some of the O blood groups, but it will strongly agglutinate other O blood groups. We

shall call the latter the O_1 type and the former the O_2 type. In other words, O blood groups can be divided into two subgroups, O_1 and O_2 . The frequency of the O_1 blood group is about 20%, while that of the O_2 blood group is about 80%. On testing the hereditary tendencies of these two subgroups with actual cases of various families, we came to the conclusion that the O_1 blood group is inherited dominantly to the O_2 blood group (Table I).

Table I
The heredity of O_1 type and O_2 type

Parents		Children		
Phenotype	Genotype	O_1O_1	O_1O_2	O_2O_2
$O_1 \times O_1$	$O_1O_1 \times O_1O_1$	1	—	—
	$O_1O_1 \times O_1O_2$	$\frac{1}{2}$	$\frac{1}{2}$	—
	$O_1O_2 \times O_1O_2$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{4}$
$O_1 \times O_2$	$O_1O_1 \times O_2O_2$	—	1	—
	$O_1O_2 \times O_2O_2$	—	$\frac{1}{2}$	$\frac{1}{2}$
$O_2 \times O_2$	$O_2O_2 \times O_2O_2$	—	—	1

O blood groups consist only of O substance, and do not contain A substance or B substance. According to the study of Inoue⁷⁾, O substance consists of the three partial antigens, O_I , O_{II} and O_{III} . The composition of the O substance varies according to the kind of animal, the simplest being $[O_{III}]$, the next $[O_{II} O_{III}]$, the O substance in human blood-corpuscles having the most complicated composition $[O_I O_{II} O_{III}]$. The O substance in the blood-corpuscles of the white rat and the kidney of the guinea pig is of the $[O_{II} O_{III}]$ type of composition.

If we immunize fowls with human O substance, we can obtain anti- O_I , the antibody against O_I , and likewise, the anti- O_{II} serum can be obtained by immunizing fowls with the blood-corpuscles of the white rat or the kidney cell emulsion of the guinea pig. From the chemical viewpoint, O substance can be decomposed into the fractions of protein (p), lipid (l) and carbohydrate (k).

On trying to find out the cause of the distinction between the two subgroups O_1 and O_2 , from the viewpoint of partial antigens, we discovered that the distinction occurred from a difference in the quantity of the O_{II} part. By making a chemical analysis, however, this difference was found to lie in the lipid fraction; in other words, the distinction between the subgroups O_1 and O_2 is determined by the difference in the quantity of the O_{II} substance in the lipid fraction.

E and e Blood Types of O Blood Corpuscles.

Sugishita⁸⁾ (1935) succeeded in dividing A, B and AB blood groups into two groups—the E-type and the e-type—through the agglutinability to eel serum, of which the E-type is dominant to the e-type. It has been commonly believed since long ago that, among the various human blood groups, the O blood group alone could never be divided into the E-type and the e-type by means of eel serum. However, we have finally succeeded in dividing human O blood groups into the E-type and the e-type.

The study of E blood-types and e blood-types began with Miyazaki's⁹⁾ report (1930) that a certain type of eel serum has a remarkably strong effect in agglutinating especially human O blood groups. This type of eel serum is called the second type, and is distinguished from the first type of eel serum which equally agglutinates all human blood groups. Taking advantage of this property of eel serum, Sugishita (1935) succeeded in dividing the human A, B and AB blood groups into AE and Ae, BE and Be and ABE and ABe respectively.

Inoue (1943), by dividing anti-O serum into anti-O_I, anti-O_{II} and anti-O_{III} sera and examining them separately, ascertained that the distinction between E and e occurred according to the action of anti-O_I, and Akimitsu¹⁰⁾ (1942) proved by chemical analysis that the cause of this difference lay in the protein fraction of O_I antigen.

Murakami allowed some eel serum of the second type to be absorbed by a appropriate quantity of some human O blood groups, and noticed that the supernatant serum had become inactive to the

Table II

The heredity of E type and e type

Parents		Children		
Phenotype	Genotype	EE	Ee	ee
E × E	EE × EE	1	—	—
	EE × Ee	$\frac{1}{2}$	$\frac{1}{2}$	—
	Ee × Ee	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{4}$
E × e	EE × ee	—	1	—
	Ee × ee	—	$\frac{1}{2}$	$\frac{1}{2}$
e × e	ee × ee	—	—	1

O blood groups which had been used in the process of absorption and e-type blood groups, but had become quite active in agglutinating some O blood groups and E-type blood groups. The former may

be called the e-type and the latter the E-type. The frequency of the OE-type is about 77%, while that of the Oe-type is about 23%. It was also made clear that the E-type is inherited dominantly to the e-type (Table II).

Furthermore, we tried to find out in which fraction lay the cause of the division of O blood groups into the two types, E and e, and discovered that it lay in the protein fraction (p) of O₁ partial antigen. This distinction between the E-type and the e-type is quite independent from the distinction between the O₁ and O₂ subgroups of O blood groups previously mentioned.

The respective frequencies of each group of O blood corpuscles are as follows (Table III):

Table III

Type	O ₁ E	O ₂ E	O ₁ e	O ₂ e
%	16 %	61 %	4 %	19 %

References.

- 1) Masaki, N., Kuwashima, N., Iseki, S., and Fukao, T.: Hanzaigaku Zasshi, **9**(4): 467-470, 1936.
- 2) Hibino, M.: Juzenkai Zasshi, **40**(9): 3574-3588, 1935.
- 3) Maekawa, H.: Nagasaki Igakkai Zasshi, **22**(7): 965-972, 1944.
- 4) Tokugawa, T.: Nagasaki Igakkai Zasshi, **24**(1): 39-43, 1946.
- 5) Morgan and Watkins: Brit. J. Experim. Path., 1448, 29, 159; cit. J. Exp. Med., **91**(4): 433-447, 1950.
- 6) Murakami, M.: Jap. J. of Legal Medicine, **5**(4): 196-208, 1951.
- 7) Inoue, Y.: Tokyo Igakkai Zasshi, **57**(2): 1-55, 1943.
- 8) Sugishita, S.: Juzenkai Zasshi, **40**(5): 1938-1963, 1935.
- 9) Miyazaki, S.: Nagasaki Idai Hoigaku Gyoho, **2**(4): 542-552, 1930.
- 10) Akimitsu, S.: Tokyo Igakkai Zasshi, **58**(1): 1-43, 1942; Hanzaigaku Zasshi, **16**(6): 564-573, 1942.