THE INHERITANCE OF ISO-HEMAGGLUTINOGENS IN RABBITS.

R. D. CAMERON AND L. H. SNYDER,
Genetics Laboratory, Ohio State University.

The search for true blood groups in animals, corresponding to the four orthodox groups in man, has not met with much success. (Snyder, 1926, 1929). In 1929, however, it was independently discovered by Levine and Landsteiner and by Fischer and Klinkhart that the blood of rabbits may possess agglutinogens for which no normal agglutinins occur. Immune agglutinins may be produced for these, however. When such immune agglutinins are produced, it is possible to separate rabbits into groups according to whether or not they possess a particular agglutinogen.

The two agglutinogens discovered by Levine and Landsteiner, and Fischer and Klinkhart, respectively, were found on comparison not to be identical. Thus, some rabbits possess both agglutinogens, some only one, some the other, and some possess neither. On this basis rabbits are of four groups.

The inheritance of these groups becomes at once of interest to the genetecist. Many genetic characters are known in rabbits, and the occurrence of a new factor makes possible the extension of linkage results. Rabbits of known group were accordingly sent by Landsteiner to Castle for genetic study. It was suggested by Castle that we cooperate in the work, the final plan being to produce the Fischer serum at Ohio State and study its agglutinogen at that institution, and to produce the Landsteiner serum at Harvard and study its agglutinogen there. In 1930 we received at the Genetics Laboratory of the Ohio State University five rabbits, two of them known to contain the Fischer agglutinogen (i.e., positive), and three known not to contain it (negative).

Our first task was to obtain a diagnostic serum. With the kind assistance of Dr. C. A. Doan we were able to produce a strong agglutinin by immunization. The method used was as follows.

Aseptic technic was used throughout. Nine cc. of blood were withdrawn from the heart of the donor (known to be

positive) into a syringe containing 1 cc. of 5 percent sodium citrate in normal saline. The donor was not anaesthetized, but was held on its right side by two assistants. With the rabbit's head at the left of the operator, the heart was located by the operator's thumb, the hair shaved from over the heart, alcohol and iodine applied, and the needle pushed between the ribs. With a little practice the needle can be inserted into the ventricular cavity and blood withdrawn.

TABLE I.

SUMMARY OF 77 FAMILIES OF RABBITS STUDIED FOR THE PRESENCE (POSITIVE) OR ABSENCE (NEGATIVE) OF AN ISO-HEMAGGLUTINOGEN.

MATINGS	OFFSPRING	
	Positive	Negative
positive x positive 40	124 calc. .880 obs. .893 dev. .003	15 .110 .107 .003
positive x negative 33	85 calc. .668 obs. .692 dev. .024	38 .332 .308 .024
negative x negative 4	0 calc. 0.00 obs. 0.00 dev. 0.00	13 1.00 1.00 0.00

The citrated blood was then injected directly into the marginal ear vein of the recipients (which were negative for the agglutinogen). The 10 cc. of citrated blood were divided among two recipients. Two donors were used alternately. The injections were made every four days until nine had been made.

After the last injection, the serum of the recipients was tested against the red cells of the donors. It was found that a strong agglutinin had been produced in the serum of the recipients. Fourteen days after the last injection, the recipients were exsanguinated, and the serum prepared and stored with a preservative.

It was then possible to test all our available rabbits for the presence or absence of the agglutinogen. A drop of a washed suspension of red cells was mixed with a drop of test serum on a slide, in a manner similar to the test for the human blood groups. The slides were examined at the end of a few minutes, and again at the end of an hour in the incubator.

In all 504 rabbits were tested; of these 379, or 75.1% were positive, and 125, or 24.9% were negative. Of these 504 rabbits, 429 were studied from the standpoint of heredity. They comprised 77 families of parents and offspring. The results of this study are given in Table I.

The agglutinogen appears to be a dominant unit character. It is seen, however, that the observed numbers of negative offspring in the crosses of positive with positive, and positive with negative, are slightly less than the calculated results. This suggests the possibility of another allelomorph.

The calculated results were obtained by the formulae

$$\left(\frac{q}{p + 2q}\right)^2 \text{ and } \frac{q}{p + 2q}$$

as developed by Snyder (1932). In the case of this agglutinogen, letting H stand for the factor producing the agglutinogen and h for its absence, and setting p as the frequency of H and q as the frequency of h, p = .502, and q = .498.

It can readily be shown that the above formulae will give the expected results if only two allelomorphs are concerned, but will give results somewhat higher than those to be expected if three allelomorphs exist. Thus in the four human blood groups, assuming that only two groups, A and O, are known (in which case group AB would be included with A, and B with O),

Let p = frequency of A
and q = frequency of O
$p^2 + 2pq$ = group A
q^2 = group O
$q = \sqrt{O}$

Since the real group O occurs with a percentage of 45 in the American population, and group with B a percentage of 10, our supposed group O will here be .45 + .10. Then

$$q = \sqrt{.55} = .741$$
$$p = 1 - q = .259$$

When, however, the four groups are actually known, due to three allelomorphs A, B and O, we may let p = frequency of A, q = frequency of B, and r = frequency of O. Then p + r, dealing with the frequencies of the factors concerned in groups A and O, will be less than 1. The derivation of p, q and r has been dealt with in previous publications (Snyder 1926, 1929 etc.).

Let R = % recessives expected in matings of A with A when only two groups, A and O, are known,

And let R^1 = % recessives expected when four groups are actually known.

Let S = % recessives expected in matings of A with O when only two groups are known,

and let S^1 = % recessives expected when four groups are known.

$$\text{Then } R = \left(\frac{q}{p + 2q}\right)^2$$

$$R^1 = \left(\frac{r}{p + 2r}\right)^2$$

$$S = \frac{q}{p + 2q}$$

$$S^1 = \frac{r}{p + 2r}$$

These formulae may be applied to the known proportions of the four blood groups and the derived frequencies of the genes in man. Using Americans and Koreans, as representing two races where the allelomorph B is low and high, respectively, the results are as given in Table II.

TABLE II.

COMPARISON OF PROPORTION OF RECESSIVE OFFSPRING TO BE EXPECTED IN VARIOUS MATINGS WHEN ONLY TWO OF THREE ALLELOMORPHS ARE KNOWN, AND WHEN ALL THREE ARE KNOWN.

	AMERICANS	KOREANS
R.....	.180	.190
R^1.....	.175	.173
S.....	.425	.436
S^1.....	.419	.416

It is seen that when only two allelomorphs are known of a series containing more than two, the recessive offspring observed in matings of dominant with dominant, and dominant with recessive, will be less than that calculated on the basis of two allelomorphs. We find this condition to exist in our results. While the differences are small, they are both in the same direction, and only a small difference is to be expected.

Inasmuch as Castle and Keeler are investigating the Landsteiner agglutinogen, and tell us that they find it to be due to a dominant factor similar to the one producing the Fischer agglutinogen, (Castle and Keeler, in press) we predict on the basis of our calculated results that the two will be found to be allelomorphs, forming with the recessive factor producing neither substance, a series of three allelomorphs, and thus four groups.

We have sent samples of our serum to Castle and Keeler for such a study, with the suggestion that matings between double positives and double negatives be especially examined as giving critical evidence on this point. Their results will be separately published.

We have also made numerous experiments on guinea-pigs, using a wide variety of donors and recipients, but have thus far been unable to demonstrate the presence of any agglutinogen in the red cells. These experiments are being continued.

SUMMARY.

1. The presence of an iso-hemagglutinogen in rabbits, originally discovered by Fischer and Klinkhart, behaves as a unit factor dominant over its absence.

2. The comparison of observed and calculated proportions of recessives in various types of matings leads us to predict the presence of a third allelomorph in the series, presumably the gene for the presence of the agglutinogen discovered by Levine and Landsteiner. This possibility is being investigated by Castle and Keeler as part of a cooperative project.

3. Attempts to demonstrate the presence of iso-hemagglutinogens in guinea pigs have thus far been unsuccessful.

BIBLIOGRAPHY.

Fischer, W., and Klinkhart, G. 1929. Arbeit. a. d. Staatinst. f. exp. Ther. zu Frankfurt a/M., **22**: 64; 1930, **23**: 65.

Levine, P., and Landsteiner, K. 1929, Jour. Immunol. **17**: 559; 1931, **21**: 513.

Snyder, L. H. 1924, Jour. Immunol. **10**: 45; 1929, Blood Grouping in Relation to Clinical and Legal Medicine, Baltimore; 1932, Ohio Jour. Sci. **32**: 436.