THE OCCURRENCE OF BLOOD GROUPS IN CATS

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(*Received* 12 *November* 1952)

(With Plate 10)

INTRODUCTION

The investigations of Ingebrigsten (1912) and of Ottenburg & Thalhimer (1915) indicated that cats could not be grouped on the basis of the isoagglutination reactions of their blood, but the experimental methods used by these workers are open to certain criticisms which make it necessary to re-examine some of their conclusions.

However, using techniques based on those suggested for use in investigations on human blood groups (Schiff & Boyd, 1942; M.R.C. memo no. 9, 1943; Wiener, 1943), it has been shown (Holmes, 1950) that there are at least two blood groups in cats: group I, those having an isoagglutinogen in the cells, forming about 97% of the population; group II, the remainder, lacking the isoagglutinogen but having the homologous isoagglutinin in the serum.

The reaction between cats' cells and sera obtained from group II animals has been used to classify members of the cat population with respect to their isoagglutinogen-isoagglutinin make-up. During such a routine grouping of a number of animals it was noticed that the red blood cells of one cat gave a delayed and distinctly weaker agglutination reaction with antiserum from group II animals than did the blood of other individuals which had been previously tested and classified as group I. A more detailed investigation of the properties of this weakly reacting blood demonstrated conclusively that it could not be classified as either group I or group II, but that a further grouping was present in the cat population. Animals belonging to this third group have blood possessing both an isoagglutinogen and an isoagglutinin.

MATERIALS AND METHODS

Adult animals, constituting a random sample of local cats, have been used throughout.

Specimens of serum were obtained by bleeding into a sterile tube from a small incision on the undersurface of the cat's ear near the edge. The blood was allowed to coagulate at room temperature (16–20° C.) for 1 hr. and at ice-box temperature (3° C.) for 1–2 hr., after which the retracted clot was removed and the serum centrifuged. The clear serum was pipetted into small, sterile glass bottles and inactivated at 56° C. for 30 min. to destroy complement and thus prevent haemolysis

* Part of this work was performed during the tenure of a Medical Research Council Studentship.

during agglutination tests. Preservation was effected by storage at −10° C. Kept in this way serum retains its strength and specificity indefinitely.

Approximately 2½% red-cell suspensions were prepared by adding one drop of blood to 1 ml. of a citrate-isotonic saline solution. Owing to the gradual deterioration of the blood suspensions only those less than 6 hr. old were used for a test.

Before the discovery of the third cat group, a quick slide method had been used for grouping unknown bloods, but since that time a tube method has been used in every case. The former consisted of agitating a slide bearing one drop of a solution of a strong antiserum (1 vol. of serum to 1 vol. of isotonic saline) tinged faint pink with whole blood; agglutination if it were going to occur showed macroscopically in from 1 to 2 min. In the tube method, performed in duplicate, one drop of a 2½% red-cell suspension of the unknown blood was mixed with one drop of diluted antiserum in a small test-tube and, after standing at room temperature for 15 min., was centrifuged at 2500 r.p.m. for 2 min. After resuspending the sediment by flicking the tube gently with the finger readings were made both macroscopically and microscopically. Adequate controls were included with every test, namely, saline plus red-cell suspensions of the three known groups; and antiserum plus red cells having no isoagglutinogen. Bloods showing no agglutination with the grouping serum and also those giving doubtful results were re-investigated by testing their sera against red cells of known group in addition to testing their red-cell suspensions with several samples of antiserum from different animals. A further check in cases of putative group II blood consisted of absorbing a serum sample with cells of the third group and testing the absorbed fluid against known cells. If the suspected blood were indeed group II, the absorbed serum still reacted with group I cells; an example of this is illustrated in Pl. 10, fig. 2.

Two agglutinin solutions have been used in routine grouping of batches of cats: (1) serum from the third group diluted with an equal volume of isotonic saline; and (2) a pure agglutinin fluid prepared as follows: ten drops of washed, packed group III red cells were mixed with 3 ml. of strong group II serum and occasionally agitated at room temperature for 1 hr. The agglutinated cells were separated by centrifuging and washed several times to remove unwanted group II sera. One and a half ml. isotonic saline was added to resuspend the sediment and the mixture heated slowly to 56° C., at which temperature it was maintained for 5 min. At 56° C. the process of agglutination is reversed, the isoagglutinin being released from the agglutinated red cells into the saline. This suspension was centrifuged in tubes surrounded with water at 56° C. for 2 min., the supernatant removed and centrifuged once again for 5–10 min. The final supernatant contained the pure isoagglutinin ready for use.

Full absorption of an isoagglutinin from an antiserum was obtained by adding 0·5 ml. of washed, packed red cells to 1 ml. of serum and centrifuging after standing for half an hour at room temperature. This process was repeated with half the volume of packed red cells used in the previous absorption, until testing the absorbed serum with the absorbing red cells gave no agglutination. Usually three absorptions were necessary to remove an isoagglutinin completely.

Titrations were performed in tubes, the antisera being diluted with saline to prepare serial twofold dilutions and a 1% red-cell suspension, prepared each time from the same group I animal, used as a standard test cell. After standing for 2 hr. the sediment was resuspended and examined for agglutination. Titres were expressed as the reciprocal of the final dilution of antiserum in the last tube showing definite microscopic agglutination.

The presence of haemolysis has been determined by means of the tube method as described but using fresh, unheated serum and incubating at 37° C. for 2 hr. Any haemolysis that occurred was easily detected by comparing the red stained supernatant with the unaffected controls. It was usual to include test cells plus inactivated serum as one of the controls.

Cold agglutinins were investigated by mixing serum separated at 37° C. with an equal volume of a 2½% suspension of washed red cells and leaving overnight at 3° C. Any sample exhibiting agglutination was incubated at 37° C. for 2 hr. during which agglutination should be reversed, for cold agglutinins are generally inactive at 37° C.

RESULTS

The results of cross-agglutinating the red cells and sera of the three cat groups are shown in Pl. 10, fig. 1. This figure was prepared by performing the tests in tubes as described and allowing one drop of the resuspended sediment to fall on the appropriate square of a cross-hatched filter-paper. After drying in the air the filter-paper retains the red-cell stains for years, the difference between agglutinated and non-agglutinated samples remaining clear and distinct. The system of nomenclature shown also in Pl. 10, fig. 1, can only be considered tentative and is used not to define clearly the agglutinogen constitution of the three groups but only as a working classification in the light of the known facts.

The reactions of cat group *O* serum with cells from groups *EF* and *F* can be most easily explained by assuming that such a serum contains at least two iso-agglutinins capable of agglutinating cells from one or both of the other groups. This supposition is supported by the results of the absorption experiments illustrated in Pl. 10, fig. 2. Absorption of group *O* sera with group *F* cells removed only one isoagglutinin, for, on testing the absorbed serum with group *EF* cells agglutination still occurs indicating that a second isoagglutinin still remains. Thus it would appear that group *O* sera contain anti-*E* and anti-*F* isoagglutinins, both of which can be removed by group *EF* cells. On this basis group *EF* has no serum isoagglutinin content while the antibody present in group *F* blood is limited to anti-*E*.

Anti-*F* isoagglutinin only occurs in group *O* sera, and here it is always present together with anti-*E*. However, by eluting the antibody from agglutinated group *F* cells a pure agglutinin fluid containing anti-*F* can be obtained. The results of testing this antibody with cells of the cat groups is shown in Pl. 10, fig. 3.

Properties of isoantibodies

Titrations of sera obtained at intervals from groups *O* and *F* cats have been performed over periods ranging from several months to 2 years. In not a single case

has the antibody strength varied markedly from its initial value. The antibodies in groups *O* and *F* sera have also retained the specificity of their reaction with cells of all groups, as demonstrated by occasional testing.

With respect to thermal amplitude both antibodies are most active at approximately 18° C., the activity falling off gradually with increase in temperature until at about 56° C. agglutination is inhibited but without destruction of the antibody. At around 3° C. antibody activity is significantly lower than at room temperature.

Haemolysins. Haemolysis regularly occurs, especially at 37° C., with fresh sera from groups *O* and *F*. However, the haemolysins responsible are only specific for *EF* cells, red cells from groups *F* and *O* under similar conditions remaining unaffected. Group *EF* sera do not appear to possess haemolysins against any cat red cells.

Cold agglutinins. Antibodies active at about 3° C. occur occasionally. They are infrequently present in blood from group *O* and from group *EF* animals and are non-specific in character, almost invariably acting as autoagglutinins of their own red cells. In all cases the cold agglutinins react weakly, never having a titre greater than 4–8 at 3° C. Blood coagulated at 3° C. invariably yielded serum which was uncontaminated by non-specific cold agglutinins, these having been absorbed by the red cells in the clot.

Recently, a weak agglutinin most active in the cold has been found in the serum of a group *EF* animal; it reacts with cells of groups *O* and *F* but not with group *EF* blood. This antibody, which reacts very weakly at room temperature and not at all at 37° C., is under investigation.

Frequency of occurrence of cat groups

Using anti-*E* and anti-*F* solutions as testing media, the groups of 103 cats have the following incidence:

Groups ...	*EF*	*O*	*F*
Absolute numbers	98	4	1
Percentage	95·15	3·88	0·97

The values for the occurrence of groups *EF* and *O* in the cat population are very similar to those reported previous to the appearance of group *F* and using group *O* serum as the single testing fluid (Holmes, 1950). In the latter case in a population of 477 cats, 97% were group *EF* and 3% group *O*.

DISCUSSION

The techinque used by Ingebrigsten and by Ottenburg & Thalhimer in their cross-agglutination experiments was based on that described by Epstein & Ottenburg (1909). In the experiments of Ingebrigsten this consisted of mixing 2 vol. of serum with 1 vol. of a 5% red-cell suspension in a capillary pipette and investigating macroscopically after 2 hr. at 38° C. and again after 24 hr. in the ice box. In this author's own words, 'The reaction was recorded as positive only when...the agglutination was plainly seen on the first as well as on the second examination. In two or three cases the agglutination was very strong and occurred some minutes

after the mixture had been made; but usually it was not marked until the next day, i.e., after 24 hours in the ice chest.' This statement suggests that Ingebrigsten may have been overlooking certain examples of true isoagglutination by not including in his protocols as positives those cases where agglutination was marked only after 24 hr. at 3° C. However, owing to the fact that Ingebrigsten gives no indication of the conditions used during the preparation of the sera, it is impossible to infer whether those examples of agglutination only noticeable after 24 hr., were due to the activity of non-specific cold agglutinins or to true isoagglutinins too weak in their reaction at 38° C. to be definitely observed macroscopically. These considerations alone make questionable whether Ingebrigsten was recording the correct results of certain of his tests and thus his conclusions must remain open to doubt.

Beyond indicating that readings were made generally after 2 hr. at room temperature Ottenburg & Thalhimer's description of the investigations into normal cat isoagglutinins omits any reference to the proportions of serum and red-cell suspension used for a test; nor do they state the concentration of erythrocyte suspensions prepared. However, in the section of their paper devoted to the appearance of haemolysins after transfusion, mention is made to 5% red-cell suspensions used in testing for haemolysins, and in an earlier paper concerned with the results of investigations into grouped isoagglutinins in rabbits and steers (Ottenburg & Friedman, 1911) the tests are described as comprising 3 vol. of serum plus 1 vol. of a 3% or 5% red-cell suspension. Thus, from these statements, it is highly probable that similar techniques were in use for the cat isoagglutinin experiments, namely, 2 vol. or 3 vol. of serum mixed with 1 vol. of an approximately 5% suspension of cells. Assuming that this is the case, let us consider some of Ottenburg & Thalhimer's findings. First, they reached the conclusion that agglutinins when present were not constant in power or specificity but varied considerably from time to time. This has not been found to be the case in the present investigation. Secondly, their statement that, 'isohaemolysins do not occur among normal cats', is also false, the presence of isohaemolysins having been clearly demonstrated. Thirdly, they find that autoagglutination sometimes occurred; moreover, the autoagglutinative sera were usually those which affected the red cells of a large number of other animals. In their protocol illustrating the results of cross-agglutination of serum and cells from fifteen animals two examples of thirty-eight recorded positives are cases of autoagglutination. Cold agglutinins have been conclusively demonstrated in the present study, and it is conceivable that the autoagglutinations observed by Ottenburg & Thalhimer were, in fact, examples of cold agglutinins still reacting weakly at room temperature. Alternatively, they could have been non-specific false reactions simulating and indistinguishable from true isoagglutination. Now, because these workers listed examples of autoagglutination among their positive reactions it is necessary to inquire, how many more out of the remaining thirty-six recorded positives were in fact cases of non-specific agglutination. In view of this it is possible that had only true isoagglutination been considered the presence of distinct groups might have been revealed. Because of this possible source of error their results cannot be accepted without reservation.

The methods used by Ingebrigsten (and most probably by Ottenburg & Thalhimer) have not given clear-cut results in this laboratory. Ingebrigsten especially did not perform his experiments under optimum conditions for the antigen-antibody reaction. Furthermore, false reactions are more liable to appear when using this technique. The methods used in the present investigations are not subject to these errors. They are based on methods which have been used so successfully in human serology and, with cat blood, they have given consistently unequivocal results in which the possibility of false reactions remaining unrecognized has been reduced to a minimum.

The results shown in Pl. 10, figs. 1–3, bear a striking resemblance to those obtainable using human sera and red cells of groups *O*, *AB* and *A* (or *B*) for the tests. Though this similarity provided a guide in choosing the tentative nomenclature of the groups no more than an analogy is claimed to exist between the reactions of the cells and sera of cats on the one hand, and the cells and sera of humans on the other. Assuming that the nomenclature shown in Pl. 10, fig. 1, does represent the actual agglutinogen content of the red cells, and bearing in mind the fact that there are four groups in man, it seems feasible that a fourth grouping exists in the cat population. If this is so, then this fourth group ought to have an agglutinogen *E* in the red cells with agglutinin anti-*F* in the sera. However, no cat group having this composition has yet been identified.

If we assume that the inheritance of the cat groups is determined by a genetical system similar to the human *ABO* model, it is possible to calculate the theoretical frequency of occurrence of group *E* in the population. Let x, y and z be the frequencies of the genes, *E*, *F* and *O* respectively, then in a homogeneous population $x+y+z=1$.

Phenotype	Genotype	Frequency
$\bar{O}$	*OO*	z^2
$\bar{E}$	*EE*, *EO*	x^2+2xz
$\bar{F}$	*FF*, *FO*	y^2+2yz
$\bar{E}\bar{F}$	*EF*	$2xy$

$$z=\sqrt{\bar{O}},$$

$$\bar{O}+\bar{F}=y^2+2yz+z^2=(y+z)^2,$$

$$y+z=\sqrt{(\bar{O}+\bar{F})},$$

or,
$$x=1-\sqrt{(\bar{O}+\bar{F})}.$$

From the observed values of groups *O* and *F*

$$x=1-\sqrt{(0{\cdot}0388+0{\cdot}0097)}=0{\cdot}7798$$

and
$$z=\sqrt{0{\cdot}0388}=0{\cdot}197.$$

Now
$$\bar{E}=x^2+2xz$$
$$=0{\cdot}7798^2+2\times 0{\cdot}7798\times 0{\cdot}197.$$
$$=0{\cdot}915 \text{ or } 91{\cdot}5\%.$$

It is obvious that the theoretical value obtained for group *E* bears no relation to the observed value. In the population considered group *E* is completely lacking, whereas if the three-gene theory is tenable this group should characterize the blood of more than 91 % of animals tested. The enormous discrepancy between the observed and calculated frequencies of group *E* suggest that the theory of triple allelomorphic genes as applied to cats does not adequately account for the known facts. Further information on this point could be obtained by breeding experiments, for, on the three allelomorphic gene theory, $EF \times O$ matings should give equal numbers of *E* and *F* offspring and no *EF* or *O*. Breeding experiments such as these are in progress but no definite conclusions are available from them at the present time.

A possible explanation of the cat data could be that a system similar to the A_1A_2O of humans is present. On this basis, E_1 would be equivalent to *EF* and E_2 would represent *F*. Group *O* serum would contain anti-*E* and anti-E_1 while group E_2 serum would have anti-E_1 only. If such a theory were indeed valid, then the group *E* postulated on the human *ABO* model would never be found. Some evidence for the E_1E_2O hypothesis is provided by the existence of an antibody present in group *EF* serum and active against groups *O* and *F* cells. It is possible that this cold agglutinin is the analogue of human anti-*O* or anti-*H* found sometimes in groups A_1B and A_1 blood.

An alternative hypothesis is that a system similar to the *Rh* system in man is operative. Here there would be two closely linked loci, one occupied by either *E* or *e* and the other by *F* or *f*. On such a theory, the absence of group *E* could be explained by the fact that the chromosome *Ef* might not exist or be so rare that its detection would be impossible. This is a similar example to the very rare chromosomes *CDE* and *CdE* of the human *Rh* system.

It is impossible to determine which of the two alternative genetical systems is actually in operation from the present knowledge. Breeding experiments could not be used as a means of differentiation for they could only help to eliminate the triple allelomorphic theory. However, a decision may be reached serologically for the demonstration of separate antibodies against *E* and *e*, and *F* and *f* would support the *Rh* model.

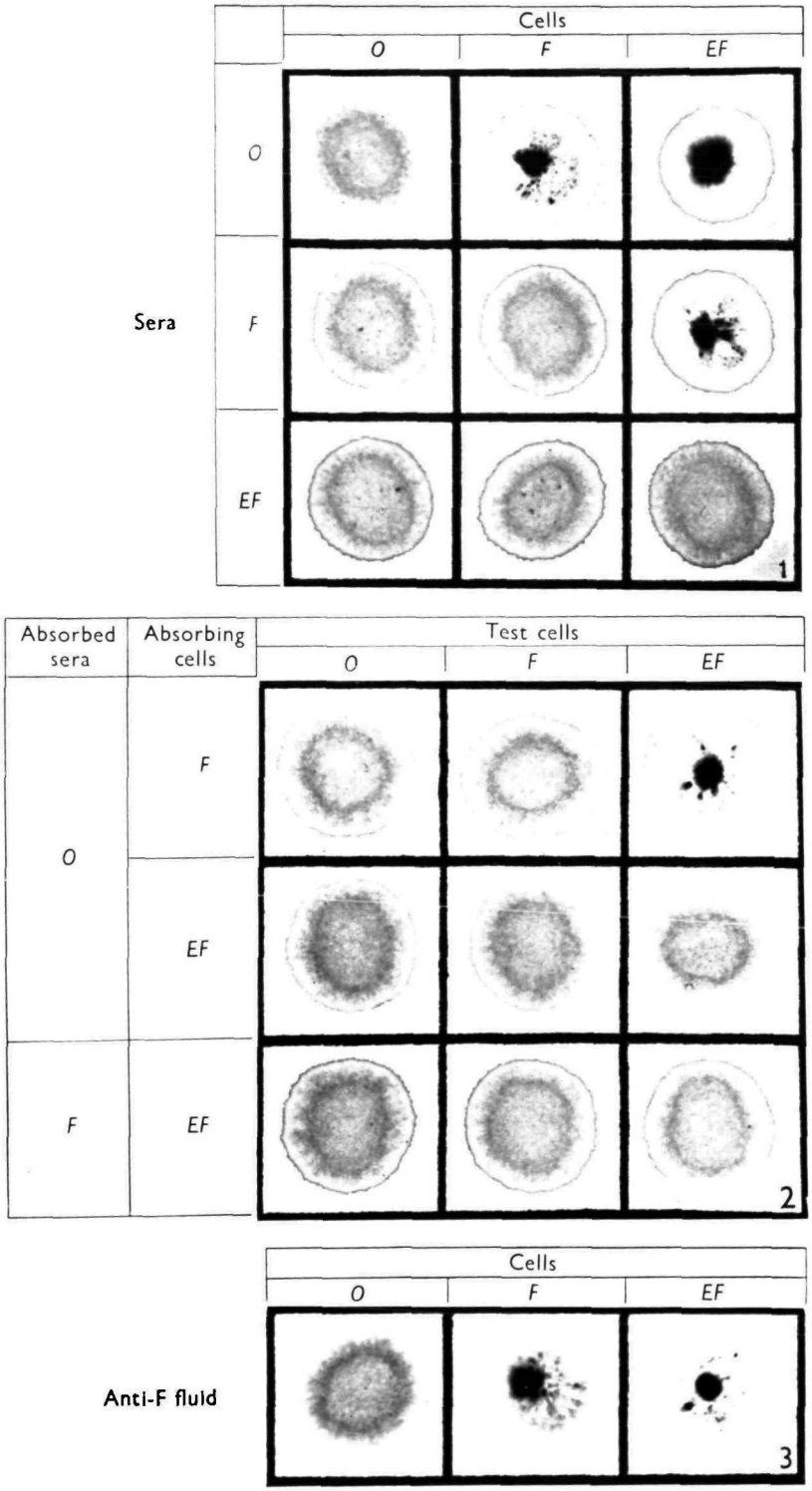
SUMMARY

Methods are described which have been used to investigate isoagglutination reactions of the blood of cats. Evidence is presented which indicates that cats possess at least three groups. The methods and results of earlier workers which indicated that grouped isoagglutinins did not occur in cats have been criticized.

A genetical system for the cat groups similar to the human *ABO* system is shown to be untenable.

Two other possible systems similar to the A_1A_2O, and to the *Rh* models of humans are suggested and briefly discussed.

I wish to express my appreciation to Dr A. E. Mourant, Director of the Medical Research Council Blood Group Reference Laboratory, for much helpful advice and criticism.



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EXPLANATION OF PLATE

Fig. 1. The results of cross-agglutinating sera and cells from the three cat groups. Black stains represent agglutination; greyish patches, non-agglutination.

Fig. 2. The results of testing absorbed cat sera with red cells from the three groups.

Fig. 3. The effect of anti-*F* fluid prepared from group *O* sera upon red cells from the three blood groups.