somes the XXYY (Muldal and Ockey, 1960) seems to have no such effect. The XXY and the XXYY Klinefelters are clinically indistinguishable from each other at present.

The genetic strength of the Y chromosome can hardly be questioned, since in the extreme Klinefelters with more than two X chromosomes, either as a single modal number or in chimeric conditions, the Y chromosome is always capable of manifesting itself to the extent of making these individuals phenotypically males, however sterile they may be. If this point is considered in terms of a polygenic system it might even be predicted that a greater material loss of Y chromatin would still swing the scales to masculinity.

The reduced size of the Y chromosome may be expected to have the effect of lowering the androgen level in the carriers—which ought to cause a reduced anabolism and in the long run bring about a condition somewhat similar to muscular dystrophy. It seems strange that two such characters should flow together in the same family, and one cannot help thinking that one must aggravate the other.

On the basis of the evidence it would appear that the male-determining genes are situated close to the centromere of the Y chromosome. Alternatively, one might envisage a system of pleiotropic masculinizing polygenes located more or less randomly along the whole length of the Y chromosome. At the moment we do not have any certain evidence of Mendelian genes on the Y chromosome (due to the lack of crossing-over between X and Y), but there are possibly genes controlling dimorphism in growth rate between the sexes, genes controlling the degree of infiltration into the embryonic gonads of primordial germ cells, genes controlling the activity of embryonic testes, and possibly genes controlling "hairy ears"—a trait which appears to be Y-linked (Gates, 1946).

The study of the deletions in the Y chromosome may enable us to localize some of these genes or, alternatively, provide evidence of a polygenic system.

Summary

A deletion of the Y chromosome has been found in a family where it occurs associated with muscular dystrophy and hypospadias. Although the evidence with regard to the muscular dystrophy does not suggest a connexion with the deleted Y, it seems most likely that an association exists between the aberrant chromosome and hypospadias.

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RED CELLS OF ALL FOUR ABO GROUPS IN A CASE OF LEUKAEMIA

BY

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A number of cases have now been described showing that certain leukaemic patients develop changes in the A antigen during the course of their disease (van Loghem *et al.*, 1957; Stratton *et al.*, 1958; Gold *et al.*, 1959; Salmon *et al.*, 1959; Salmon and Bernard, 1960; Hoogstraten *et al.*, 1961). The change observed is that the red cells become relatively inagglutinable by anti-A blood-grouping sera. When the patients' cells have been tested initially their blood group has often been difficult to determine because little or no agglutination may occur with anti-A sera. Commonly there has been only a partial (or mixed field) agglutination—that is, both agglutinates and free cells were present though the proportion of each differed from case to case. Examination of the bone-marrow cells has yielded similar results (van Loghem *et al.*, 1957; Stratton *et al.*, 1958).

In some cases records were available of blood-grouping tests carried out prior to the onset of the disease, and the patients were found to be normal group A in these tests (van Loghem *et al.*, 1957; Stratton *et al.*, 1958; Hoogstraten *et al.*, 1961). The suggestion that their A antigens were originally normal was supported by the family study in our previous case (Stratton *et al.*, 1958), which showed that the patient was genetically A₁, and by two other cases in which the family study was suggestive of the presence of a normal A gene (Gold *et al.*, 1959; Salmon *et al.*, 1959). In many of the cases described the change was confined to the A antigen, and the antigens of the other blood group systems appeared to be normal, though changes have been found in the H and Le^a antigens (Tovey, 1959), in the B, H, M, N, and D antigens (Májský and Brabec, 1961), and in the D and E antigens (Tovey *et al.*, 1961).

Although serologically the changed red cells which these leukaemic patients possess when they present themselves for examination are difficult to classify, nevertheless it is clear from these cases that there has been a change in the A antigen on the patients' erythrocytes during the time they were suffering from leukaemia.

Gold *et al.* (1959) showed that there was a partial reversion towards normal in the patient's red cells during a temporary clinical remission. In another case also the patient's cells reverted to normal A₁, but this was in the terminal stage of the disease (Hoogstraten *et al.*, 1961).

The significance and cause of these changes in the A antigen has not been clear, and we thought when we studied our first case that an enzyme produced by the leukaemic white cells might be affecting the A antigen on the red cells, although attempts to reproduce the phenomenon *in vitro* were unsuccessful. In the present case, therefore, the object of the study was to try to

elucidate the nature of the changes that occurred in the patient's A antigen. The patient proved to be group AB, and our studies enabled us to show that transfused cells from a group A₁ donor did not sustain any change to the A antigen while in the patient's circulation, although detailed studies of the patient's own red cells showed that he had changes in both A and B antigens.

Case History

A man aged 66 was admitted to the Manchester Royal Infirmary suffering from breathlessness, oedema, and loss of weight for the preceding nine months. He was a retired coal-miner and was already known to be suffering from pneumoconiosis. He had an enlarged spleen and his blood count was as follows: haemoglobin, 52% (Haldane); R.B.C.s, 3,500,000/c.mm.; platelets, 380,000/c.mm.; W.B.C.s, 69,000/c.mm. Examination of the peripheral blood smear revealed a predominance of eosinophils and eosinophilic metamyelocytes, together with some myelocytes and 11.5% "blast" cells. Many nucleated red cells were present and anisocytosis and poikilocytosis were marked. The bone-marrow showed a predominance of eosinophilic granulocytes, together with a number of myelocytes and myeloblasts. A diagnosis of eosinophilic leukaemia was made.

His blood groups were found to be: (AB), R₁r, MN, S+, P+, Lu(a+), K-, Le(a-b-), Fy(a+). No blood-group antibodies were detected in his serum, and no incompatibility was found in cross-matching tests prior to his transfusion. Paper electrophoresis of the patient's serum gave a normal pattern, and the percentage of foetal haemoglobin was within normal limits for an adult.

He was treated with busulphan ("myleran"), ferrous gluconate, and blood transfusion, but, although his white-cell count fell to 20,000 and his haemoglobin rose to 70%, he developed pneumonia and died 39 days after admission.

At necropsy the existence of pneumoconiosis was confirmed. The marrow and spleen were packed with eosinophils and there was also eosinophilic infiltration of the myocardium. The post-mortem diagnosis was eosinophilic leukaemia, bronchopneumonia, and pneumoconiosis.

When the patient had been treated with busulphan for eight days his ABO group was determined prior to transfusion. It was found that a mixture of agglutinated and unagglutinated cells was obtained when his cells were tested with anti-A serum, and a similar phenomenon was observed with anti-B. His serum failed to agglutinate A and B cells. This suggested changes in both the A and B antigens similar to those described in certain other cases of leukaemia, and the case was therefore studied with a view to elucidating the nature of these changes.

Tests on Cells Recovered from the Patient's Circulation After Blood-transfusion Therapy

The patient, who was R₁r, K-, was transfused as follows:

Day 0: Packed cells of two bottles, group A₁, rr, K+, given (these had been stored as whole blood for six and four days respectively).

Day 5: First post-transfusion sample taken. Packed cells of two bottles, group A₁, rr, K+, given (these had been stored as whole blood for one and three days respectively).

Day 12: Second post-transfusion sample taken, and further transfusion given.

The patient died eight days later and no further samples were obtained.

The percentage of transfused cells in the first post-transfusion specimen was estimated by a differential agglutination method based on the technique of Ashby (1921). With the use of anti-C + D, 31% of the patient's cells were shown to be transfused ones, and with anti-K, 32%; which is in good agreement with the number expected.

Experiments were next carried out in order to determine whether the A antigen of the transfused cells had been modified in the patient's circulation. Cells reacting with anti-A₁ were present in the first post-transfusion sample, and also in greater numbers in the second sample, though the actual numbers present could not be counted. With the use of incomplete anti-D and Coombs reagent, the D-negative cells—that is, the transfused cells—were separated from the second sample and found to react as normal A₁ cells when tested with anti-A and anti-A₁, though the titration score with anti-A was a little lower than that given by fresh A₁ cells. It was thought that the age of the cells (up to six days stored in ACD and up to 12 days in the patient's circulation) was sufficient to account for this. The effectiveness of the separation was confirmed using anti-K.

The absence of any alteration to the A antigen of the transfused cells was confirmed by two further experiments. In the first it was shown that α free cells—that is, inagglutinable cells separated from a suspension of the patient's cells treated with anti-A—prepared from the first post-transfusion sample were completely agglutinated by anti-D but completely inagglutinable by anti-K, showing that none of them were transfused cells, and thus that none of the transfused cells had become inagglutinable by anti-A. The second experiment consisted in treating the patient's blood with anti-A and counting the unagglutinated cells. These free cells could be entirely accounted for by the patient's own cells on the hypothesis that the transfused cells gave no more free cells with anti-A than they did before transfusion.

It was concluded from these experiments that the A antigen of the transfused cells remained unaltered in the patient's circulation.

Analysis of Patient's Erythrocytes

A sample of the patient's blood, obtained before he had received any transfusions, was subjected to serological analysis.

The initial observation was that his erythrocytes were only partially agglutinated by both anti-A and anti-B sera. Both agglutinates and many free cells were found in these tests, though control tests using these sera against normal A₂B cells gave only a few free cells using the anti-A sera and scarcely any using the anti-B. The patient's cells were not agglutinated by anti-A₁, by group AB sera, nor by his own serum and the direct Coombs test was negative.

No anti-A or anti-B was detected in the patient's serum and his saliva contained both A-substance and B-substance, so that it was thought that his true group was AB. The family studies confirmed this and showed that his true group was A₁B (see below).

Selected group O sera agglutinated a much higher proportion of the patient's cells than did anti-A (group B) sera; some gave very few unagglutinated cells, and one almost none, so that it might be thought that A₂ cells were present. However, it has been shown that group O donor cells can acquire sufficient A antigen when in the circulation of a group A recipient to become agglutinable by group O sera (Renton and Hancock, 1962). It was not possible to distinguish between A₂ cells and O cells with acquired A antigen in the present case, so that the reactions with group O sera were not investigated further.

The patient's red cells and normal A₂B cells were compared by titration and absorption of anti-A and anti-B. The results indicated that the patient's A antigen resembled that of the normal A₂B blood, although the B antigen was weaker. Absorptions of anti-A and anti-B with leucocyte suspensions prepared from the patient and from normal A₂B blood did not remove more antibody than could be accounted for by the contaminating red cells in these suspensions.

Attempts to produce similar changes in normal A₂B cells *in vitro* were unsuccessful. A₂B cells, both as washed cells

and as citrated whole blood, were incubated with suspensions of the patient's white cells in the patient's serum for varying periods up to two hours at 37° C., but no change in their agglutination reactions with anti-A or anti-B was found. Separation of the older and younger red cells from the patient's blood by the differential centrifugation method of Constandoulakis and Kay (1959) did not result in any difference in the agglutination reactions of the separated fractions when these were tested with anti-A and anti-B.

An experiment was carried out in order to determine whether those cells which had lost their A antigen had also lost their B antigen, or whether the two antigens were affected independently of one another. The patient's cells were treated with anti-A and the agglutinates separated from the free cells by differential centrifugation. The agglutinated cells were then disagglutinated by shaking with A-substance followed by washing. These cells are subsequently referred to as "α disagglutinated cells." The free cells were also washed, and are referred to as "α free cells." β disagglutinated and β free cells were prepared similarly.

These cells were then tested with anti-A, anti-B, and anti-H (*Ulex europaeus* extract), and the percentage of free cells in the tests with anti-A and anti-B was determined by differential agglutination. The results of this experiment are shown in the Table. It was at once apparent that previous treatment with anti-A did not affect the results with anti-B, and vice versa. This shows that the loss of the A antigen is independent of the loss of the B antigen. The experiment

and β disagglutinated cells tested with anti-A in exactly the same way, to give O=22.1%, A=10.9%, B=40.2%, AB=26.8% of the whole.

The mean of these two independent determinations of the percentages is, O=20%, A=12%, B=42%, AB=26%.

The supposition that cells of all four ABO groups were present was confirmed by separating cells of each group from the patient's blood. The scheme of separation is shown in the Diagram. The separated cells gave the same reactions as normal A, B, AB, and O cells when tested with anti-A and anti-B, and no mixed agglutination was seen in any of the tests.

Scheme of separation of the four ABO groups from the patient's blood.

The Rh, MN, S, P, Lu^a, K, Le^a, Le^b, and Fy^a groups of the separated cells of the four ABO groups were determined and found to be identical, though minor differences of reactivity in some of these groups could have escaped notice with the antisera used.

Family Study

The groups of the patient and his family were:

Patient: (AB) R₁r MN S + P + Lu(a+) K - Le(a-b-) Fy(a+)
 Wife: O rr MN S + P + Lu(a-) K - Le(a-b+) Fy(a-)
 Son: B R₁r N S - P + Lu(a+) K - Le(a-b+) Fy(a+)
 Son: A₁ R₁r MN S + P - Lu(a+) K - Le(a-b-) Fy(a+)
 Son: B R₁r MN S + P - Lu(a+) K - Le(a-b+) Fy(a-)
 Son: B rr M S + P + Lu(a+) K - Le(a-b+) Fy(a-)
 Daughter: B R₁r MN S + P - Lu(a+) K - Le(a-b-) Fy(a-)
 Son: A₁ R₁r MN S + P - Lu(a+) K - Le(a-b+) Fy(a-)

It is clear that the patient's genotype is A₁B.

Discussion

Changes in the A antigen in leukaemia resulting in partial agglutination with anti-A have been observed on a number of occasions, though the origin of these changes has not been clear. In the present case, however, no change was found in the antigen of normal A₁ cells retrieved after a period of 12 days in the patient's circulation, and in particular the reaction of the transfused cells with anti-A₁ was unchanged. Had anything affected the A antigen of these transfused cells we would expect a loss of reaction with anti-A₁ as the first sign of this, so that it seems unlikely that the changes in the patient's A and B antigens were due to the effect of any agency on his mature erythrocytes. The family study makes it clear that the patient was group A₁B in the first place, and tests carried out before the patient had received any transfusions showed that erythrocytes of groups A₂B, A₂, B, and O were present in his blood, but no A₁B or A₁ cells were detected. It is thought that such a situation can arise only in the chromosomes, since it is only at the chromosome level that A is separate from B in an AB individual. Treatment of the patient's cells

Reactions of α and β Free and Disagglutinated Cells

	Anti-A			Anti-B			Anti-H
	Agglutination	% Free Cells	% Agglutinated Cells*	Agglutination	% Free Cells	% Agglutinated Cells*	
α disagglutinated cells ..	+++++	3	97	+++++	35	65	+++
α free cells ..	-	98	2	+++++	30	70	+++
Untreated cells ..	+++	61	39	+++++	33	67	+++
β free cells ..	+++	67	33	-	90	10	+++
β disagglutinated cells ..	+++	60	40	+++++	2	98	+++

* 100 minus % free cells.

α free cells=cells which remain unagglutinated after treatment with anti-A.
 α disagglutinated cells=cells which have been agglutinated by anti-A, and the separated agglutinates disagglutinated by A-substance.

also showed that cells of all four ABO groups were present in the proportion of 20% O cells, 12% A cells, 42% B cells, and 26% AB cells. The method of calculation is as follows:

Thirty-nine per cent. of the untreated cells are agglutinated by anti-A, and 61% are not agglutinated, so that the α disagglutinated cells—that is, the A and AB cells—are 39% of the whole, and the α free cells (O and B) are 61%.

Sixty-five per cent. of the α disagglutinated cells are agglutinated by anti-B and these are the AB cells, and 35% are not agglutinated and are the A cells.

The AB cells are thus $65\% \times 39\% = 25.4\%$ of the whole, and the A cells are $35\% \times 39\% = 13.7\%$.

Similarly from the results with the α free cells we get O cells = 18.3%, B cells = 42.7%.

Now the percentage agglutination of untreated cells by anti-B can be combined with the four results for β free

with anti-A did not affect their reaction with anti-B, and vice versa, so that the changes in the A and B antigens appear to be quite independent of one another, and it is extremely difficult to imagine any enzyme—or indeed any other agent—which can affect AB cells in this way.

Morgan and Watkins (1956) showed that the group-specific substance in the secretions of group AB people is not a mixture of separate A- and B-substances but a single AB substance. Although it is not known whether this applies to the lipoidal group-specific substance of the red cells, this might well be so, and would make it even more difficult to imagine any agent which could change some AB cells to A and others to B.

Thus the results of the study of transfused cells and of the patient's own cells were complementary and led to the conclusion of chromosomal changes in the red-cell precursors, and it was of interest that a large number of stem cells were present in the patient's bone-marrow and peripheral blood. If chromosomal changes occurred in the stem cells one could imagine that some would develop into erythrocytes with defective antigens and others into leukaemic leucocytes.

In the case described by Gold *et al.* (1959) the proportion of cells which were inagglutinable by anti-A diminished during a remission but increased again during a relapse. This change, however, need not be regarded as contrary to the idea of chromosome abnormality, since the remission was the result of treatment with 6-mercaptopurine and prednisone, and it might be supposed that this had interfered with the proliferation of abnormal stem cells to a greater extent than with that of normal ones. It is, however, less easy to understand why the red cells in the case described by Hoogstraten *et al.* returned to normal A₁ in the terminal six weeks of the illness.

Our patient's blood contained 20% O, 12% A, 42% B, and 26% AB cells, and this means that 62% of his cells had lost their A antigen and 32% their B antigen, and it is interesting that the proportion of O cells present (20%) was equal to 62% × 32%. This, of course, may be fortuitous, though 6,600 cells were counted in these experiments and it was thought that the percentage of O cells found was unlikely to be more than 3% in error. If it is not fortuitous it supports the idea that the O cells are the end-result of two independent processes, and it also suggests that the precursors of all the patient's erythrocytes had been exposed to damage, since the presence of normal AB cells which had not been involved in the leukaemic process would have given a different set of percentages.

The idea that all the erythrocytes present might be of abnormal origin is supported by a consideration of the A₁-A₂ subtypes. The patient's genotype from the family study was A₁B, yet no A₁B or A₁ cells were found in his blood, though tests on mixtures of A₁B and A₂B cells showed that as few as 2% of A₁B or A₁ cells would have been easily detected if they had been present, and there was no difficulty in detecting A₁ cells in his circulation after transfusion with A₁ blood. The fact that transfused A₁ cells reacted normally with anti-A₁ after 12 days in the patient's circulation suggests that the loss of the A₁ antigen took place at the chromosome level.

It is of interest that in many of the cases of this phenomenon which have been recorded only the ABO groups have been affected, though changes in the antigens of some of the other blood-group systems have

been described, and it would seem that changes in the A and B antigens would, for technical reasons, be easier to detect than changes in the other antigens.

Summary

The red cells of a patient suffering from eosinophilic leukaemia, and known from family studies to be of genotype A₁B, were only partially agglutinated by anti-A and anti-B. The abnormality of the A antigen was independent of the abnormality of the B antigen, and the patient's blood was shown to contain A₂B, A₂, B, and O cells but no A₁B or A₁ cells. Similar changes could not be induced in normal red cells *in vitro*, nor in the patient's circulation.

These findings can only be explained by supposing that changes had taken place in the chromosomes concerned with the ABO groups.

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Preliminary Communications

Dermal Leishmaniasis in British Honduras : Transmission of *L. brasiliensis** by *Phlebotomus* Species

Phlebotomus species have long been the primary suspects concerned in the transmission of leishmaniasis wherever the disease occurs throughout the Old and the New World. The geographical distribution of this insect coincides with that of leishmaniae, and infected *Phlebotomus* have been found in nature. Furthermore, leishmaniae have been experimentally transmitted to volunteers by these insects (Adler and Theodor, 1957, review). Nevertheless, experimental transmission has always remained difficult to achieve, and we feel that the present observations on the transmission of *Leishmania brasiliensis* by *Phlebotomus* in British Honduras are worthy of note. Clinical and other aspects of cutaneous leishmaniasis in this region of Central America have already been discussed by Biagi F. (1953) in Mexico and by Garnham and Lewis (1959) in British Honduras.

**Leishmania tropica mexicana* is the taxonomy preferred by some authors.