It follows that anastomoses between dizygotic human twins either must be very rare or must develop at a time when the ancestral blood cells are losing their faculty to take root in a foreign bed, or when the host tissue is becoming less tolerant of immigrant cells. Professor J. D. Boyd thinks that anastomoses are rare but probably not as rare as the very infrequent recognition of chimeras would suggest. In monozygotic twins, on the other hand, mixed blood must be very common—though undemonstrable.

Judging by the W. twins it seems that, in man, the proportion of the two kinds of blood in one twin is independent of the proportion in the other twin. This is not what happens in cattle, for according to Irwin (1955) each of a pair usually has the same mixture; that is to say, if twin A has 60% A blood and 40% B blood then his fellow twin B will also have 60% A blood and 40% B blood.

Dr. Davidson's finding of female "drumsticks" in the nuclei of some of the polymorphs of the male twin shows that the ancestors of these cells too can be successfully grafted. The proportion of polymorphs with "drumsticks" was compatible with a graft of the extent shown by the red cells; if this proves a general rule it may have useful implications in the study of the lineage of these cells.

The female twin is of the genotype *OO*, and 99% of her red cells are group O. Her serum contains anti-B but no anti-A. The absence of anti-A is presumably a manifestation of the phenomenon of acquired tolerance, studied particularly by Billingham, Brent, and Medawar (1953, 1956); the presence of A cells early in uterine life has presumably inhibited the production of anti-A.

In cattle and in fowls the tolerance to foreign blood cells acquired in foetal life can later be shown not to be limited to blood cells: the majority of dizygotic twin cattle are fully tolerant to grafts of each other's skin (Anderson, Billingham, Lampkin, and Medawar, 1951), and the same is true of chickens (Billingham *et al.*, 1956). Professor Medawar and his colleagues think it probable that skin could be successfully grafted between Miss W. and her twin brother.

Both twins are secretors. They secrete the ABH antigens for which they have inherited genes: they do not secrete the antigens of their grafted red cells. From this we may assume that the ABO genes do their parallel work of manufacturing water-soluble A, B, and H substance in cells other than those in which they are busy providing alcohol-soluble A, B, and H substance for the red cells.

The first human chimera to be recognized, Mrs. McK, has demonstrated conclusively, by having three children, that the hormones of a human male foetus had no power to upset the normal sexual development of his female twin. In the parallel situation a cow is sterile. Evidently the human male is backward, compared with the bull, in developing his endocrines—which is perhaps not surprising considering the relative tempo of their two lives. In man we suppose that the embryonic female hormones have no effect on the development of the male twin—but on this point there is so far no evidence.

Summary

Two human blood chimeras are described—twins of different sex. The male twin has 86% A_1 and 14% O red cells; he also has female "drumsticks" in some of his polymorphonuclear white cells. The female twin has 99% O cells and 1% A cells.

The A_1 cells are *MSMs, CDe/cde, Fy^bFy^b, Jk^aJk^b*; the O cells are *MSMS, cDE/cde, Fy^aFy^b, Jk^aJk^a*; in their other groups they do not differ. Secretion tests on saliva show that the A_1 series belongs genetically to the male twin and the O series to the female.

We are grateful to the blood donor, Miss W., and to her family, who have been most generous in their help. We are indebted to Dr. Amos Cahan, of the Knickerbocker Foundation, New York, for a large supply of A substance.

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HUMAN BLOOD CHIMERAS

A STUDY OF SURVIVING TWINS

BY

J. W. NICHOLAS, M.R.C.S., L.R.C.P., D.C.P.
Pathology Laboratory, Essex County Hospital, Colchester

W. J. JENKINS, M.D.

AND

W. L. MARSH, F.I.M.L.T.
North-East Metropolitan Regional Blood Transfusion Centre, Brentwood, Essex

The term "chimera" (originally a fabulous fire-spouting monster, part lion, part goat, and part serpent) has been used in botany for many years to denote an individual plant in which there are two or more tissues differing in their genetic constitution, such as a graft hybrid. Its first application in mammalian physiology appears to be that of Anderson, Billingham, Lampkin, and Medawar (1951), who applied it to the condition in cattle mentioned below.

It has long been known (Owen, 1945) that communication between the circulations of binovular bovine twins can occur *in utero*, resulting in an interchange of erythroblasts with persistence throughout life of mixed blood groups. The only such human chimera hitherto reported has been that of Dunsford, Bowley, Hutchison, Thompson, Sanger, and Race (1953), in which, unfortunately, one twin died in infancy. We now present a study of twins both of whom are chimeras and whose relatives were available. Simultaneously with this, another pair of chimeras is being reported by Booth, Plaut, James, Ikin, Moores, Sanger, and Race (1957).

The propositus, Mrs. W., is a healthy married woman aged 29 who attended for antenatal blood grouping in July, 1956. She has now had three pregnancies resulting in healthy children. When her blood was tested with anti-A serum moderate-sized agglutinates were seen in a sea of free cells. Further investigation showed her blood cells to be of two distinct groups. Her twin brother, both parents, husband, and all her three children were studied. The brother, as was first shown by Dr. Dorothy Parkin, is also a chimera. Neither of the twins has ever had a blood transfusion.

Methods

The mixture of cells in each chimera was separated by differential agglutination, using anti-A and group O sera in parallel, and the proportion of free group O cells determined by triplicate counts in a haemocytometer. Deposited group A cells were freed of the α-agglutinin by elution at 56° C., but then gave equivocal results with most antisera.

The A cells were also separated by differential agglutination with a potent anti-M serum, but became panagglutinable in human serum, having presumably been coated with incomplete anti-human species agglutinins. The genotype of the A cells was therefore largely deduced by comparison of the results of mixed-field agglutination with those obtained on the separated O cells. Dr. Race, Dr. Sanger, and Miss Moores also separated the A cells, using the method described by them (Booth *et al.*, 1957), and confirmed our deductions. We have since been able to elute the human anti-A used for our separation by a technique similar to theirs but using A-secretor saliva. We have checked the results on Mrs. W. on the A cells thus separated.

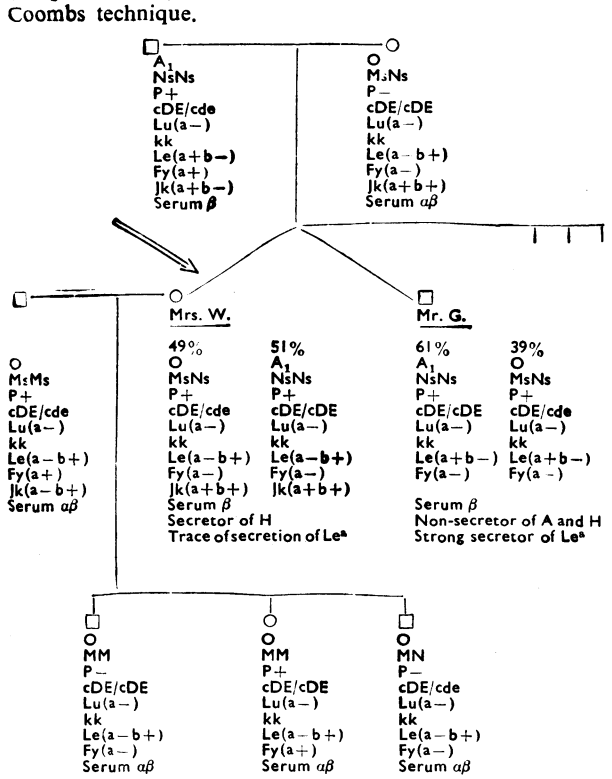
The antibodies used for grouping were : anti-A, A_1, B, A+B, H ; anti-M, N ; anti-P ; anti-C, D, E, c, e ; anti-Lu^a ; anti-K ; anti-Le^a, Le^b ; anti-Fy^a. *Ulex europaeus* anti-H and rabbit anti-M and N sera were used, other sera being of human origin. Appropriate controls were employed throughout. Kidd and S grouping was done by the M.R.C. Blood Group Research Unit, who also checked the Lewis groups, using two anti-Le^a and two anti-Le^b sera.

In testing for Le^a substance serial doubling dilutions of saliva were mixed with one volume of diluted anti-Le^a serum and left at room temperature for 30 minutes. One volume of Le(a+) cells in albumin was then added and the mixture examined microscopically after two hours' incubation. The results are shown in the Table. Standard

Saliva Dilutions Inhibiting Anti-Le^a, Anti-H, and Anti-A

Saliva	Anti-Le^a	Anti-H	Anti-A
Control group O Le(a+) ..	1 in 64	No inhibition	No inhibition
,, O Le(a−) ..	1 ,, 2	1 in 32	,, ,,
Mr. G.	1 ,, 128	No inhibition	,, ,,
Mrs. W.	1 ,, 2	1 in 32	,, ,,

quantitative techniques were used for estimation of A and H substances in saliva. The sera of the family were tested against a panel of fully grouped cells covering the ABO, MNS, P, Rhesus, Lutheran, Kell, Lewis, Duffy, and Kidd groups in saline, serum/albumin (1:1), and by the indirect Coombs technique.



Blood groups of the twins and their family. The genetic groups of Mrs. W. and Mr. G. are shown immediately below their names, the foreign cells in the column to the right. ⟹ = Propositus. Rhesus groups are probable genotypes.

Coverslip films of capillary blood were made and examined for nuclear sex of the neutrophils by Dr. W. M. Davidson (see Davidson and Smith, 1954).

Results

The results of investigating the eight members of the family are shown in the pedigree (see Fig.). Each of the twins has blood of two distinct types, differing in ABO, MN, and Rhesus groups. All Mrs. W.'s cells are, however, Le (a−b+) and all Mr. G's Le (a+b−). The twins are of different secretor status ; the Table shows their saliva reactions. The sera of both twins contain anti-B agglutinin to a titre of 1 in 64. Tests at various temperatures in saline, serum/albumin, and using anti-human globulin serum failed to demonstrate anti-A either free in the serum or blocking or coating the cells. No irregular antibodies were detected. The cells and sera of the remaining six members of the family showed no abnormality.

Nuclear sexing of the neutrophils revealed six "drumsticks" in 318 cells in Mrs. W.'s blood, and six in 338 in Mr. G.'s. Mrs. W.'s Arneth count was 3.27 and Mr. G.'s 3.30.

Discussion

There can be no doubt that the mixed blood groups arise from the interchange of erythroblasts *in utero*. The only alternative theory, that of somatic mutation, is excluded by the occurrence of a similar condition in both twins and the involvement of more than one blood group system and of the leucocytes.

Mrs. W.'s husband is of group MM and two of her children are of this group. It therefore follows that her genetic group must contain an M gene, and is therefore the O series given directly under her name in the Fig. This is confirmed by the fact that she is a secretor of H but not of A. Mr. G.'s genetic group cannot be determined with certainty as he is a non-secretor of ABH substance, and is unmarried. It seems reasonable, however, to presume that it is A_1, thus accounting for the A_1 cells in Mrs. W.'s blood.

It is now well established (see Race and Sanger, 1954) that Le(a+) individuals are all non-secretors of water-soluble ABH substance and secretors of Le^a, and that Le(a−) individuals are nearly all ABH secretors with only a trace of Le^a in the saliva. The secretor status of the twins corresponds to the Lewis groups found. Sneath and Sneath (1955) have shown that red cells take the Lewis group of the plasma in which they are suspended. This was demonstrated by transfusion experiments and also *in vitro*, and they suggest that the Lewis antigens are adsorbed to the cells from the plasma. Stormont (1949) has shown that the antigens of the J group of cattle behave similarly, and also that interchange of blood groups in bovine chimeras does not include the J group. The foreign cells in the circulation of each of the twins we have described have adopted the Lewis group of the host. Our results therefore bear out Sneath and Sneath's conclusions that the Lewis antigen belongs to the tissues and is only secondarily attached to the red cells ; they also closely parallel Stormont's findings.

Our findings on Mrs. W. support the view that the early existence of group A cells has suppressed the formation of anti-A. It is of interest that Mr. G. has not produced anti-M or anti-e in response to the foreign MNEe cells. Although Mrs. W. is a secretor and although her blood contains 51% of borrowed group A cells she is not able to secrete A substance in her saliva.

We have so far shown the presence of foreign red cells in each twin. Foreign white cells of the "chromatin-positive" type normally found only in the female are also present in Mr. G.'s blood. It is probable that foreign leucocytes are present in Mrs. W., though it is more difficult to be certain in her case. On the basis of her Arneth count the number of chromatin-positive cells is rather low. The fact that the number of chromatin-positive leucocytes is similar in both twins is in keeping with our finding of roughly equal proportions of red cells of the two types in

each twin. Because of the presence of foreign leucocytes it seems more appropriate to call these people blood chimeras than blood-group chimeras. We have found no evidence of hormonal effects; Mrs. W. is a normal healthy woman with three healthy children and therefore not a freemartin.

There are two pairs of twins among first cousins, but one of each pair died in infancy. Attempts have been made to investigate the survivors, but so far without success.

Summary

A pair of binovular twins is described, each of whom has blood which is a mixture of the same two distinct types differing in ABO, MN, and Rhesus groups. Each twin has, however, only a single Lewis group, and the Lewis groups of the twins are different, each corresponding to that expected from the secretor status. A family study of the blood groups has been made. Nuclear sexing of neutrophils shows that foreign leucocytes are present in the blood of the male twin and probably present in the female twin.

It is considered that these are chimeras similar to that reported by Dunsford *et al.* The Lewis group findings confirm the demonstration by Sneath and Sneath that the Lewis antigen belongs to the tissues and is only secondarily attached to the red cells. Results in the Lewis group are comparable with those in the J group of bovine chimeras.

We are indebted to Mrs. W. and all her family for their co-operation in this investigation; to Dr. Dorothy Parkin, of the Medical Research Council Blood Group Reference Laboratory, for some of the preliminary investigations of the twins, and also for first demonstrating that Mr. G. is also a chimera; to Dr. R. R. Race, Dr. Ruth Sanger, and Miss Phyllis Moores, of the Medical Research Council Blood Group Research Unit, for checking our results, determining the Kidd and S groups, and examining A cells separated by their technique. We are also grateful to Dr. Race for much valuable advice and to Dr. W. M. Davidson, of King's College Hospital Medical School, for undertaking the nuclear sexing.

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Medical Memorandum

Leukaemia and Oestrogen Therapy

Oestrogens have been shown by many workers to produce neoplasms in experimental animals. The risk of producing or accelerating neoplastic growth in human therapy is usually thought to be entirely hypothetical, provided that the dosage is within the usual therapeutic limits, which is much less than that used in experimental work.

Kirschbaum (1951), Burrows and Horning (1952), and Gardner (1953) have reviewed the literature on the effects of oestrogens on the incidence of leukaemia in mice. It is clear that, experimentally, oestrogens do increase the incidence of leukaemia in animals and that this is of the lymphatic type.

No record has been found of human lymphatic leukaemia associated with oestrogen therapy. A case is recorded in which lymphatic leukaemia occurred during stilboestrol therapy for carcinoma of the prostate.

CASE REPORT

A man aged 70 was seen as an out-patient in March, 1953, with symptoms of frequency and dysuria, said to be of six weeks' duration. The prostate was not enlarged, but was hard and nodular. The other findings relative to our story were that the liver was not palpable and that no abdominal mass was felt, indicating that the spleen was not palpable. Some posterior cervical glands were noted. Radiological examination revealed no metastases in spine, pelvis, and skull. The blood count on April 1 showed: haemoglobin, 86% (12.6 g./100 ml.); R.B.C., 4,410,000 per c.mm. A leucocyte count was not made. The formaldehyde stable acid phosphatase was 35 units per 100 ml. A diagnosis of prostatic carcinoma was made, and stilboestrol therapy was begun. He was treated as an out-patient. About the middle of April 10 mg. thrice daily was prescribed, and during the next few months he had 4,000 mg.

On September 19 his general practitioner observed marked oedema of the legs and an enlarged spleen. He was referred to the out-patient department, and the blood count on September 29 was: haemoglobin, 55% (8.1 g./100 ml.); R.B.C., 2,800,000 per c.mm.; formaldehyde stable acid phosphatase, 0.8 unit per 100 ml. A leucocyte count was not done.

On October 31 he was admitted to hospital with severe symptoms of anaemia. Blood count on November 1 showed haemoglobin, 28% (4 g./100 ml.); R.B.C., 1,620,000 per c.mm. Leucocytes 44,000 per c.mm. (lymphocytes and smear cells 95%, polymorphs 5%). Spleen was enlarged to the level of the umbilicus. Blood transfusions were given, and the degree of anaemia fluctuated accordingly. The total leucocyte count increased to 70,000 on November 12 and fell to 38,200 on the 16th, with no change in the type of cell present. Marrow aspirated from the ilium on November 10 showed the appearances typical of lymphocytic leukaemia. Death occurred on November 21.

At post-mortem examination enlarged glands were found in the neck, mediastinum, mesentery, and around the aorta and common iliac arteries. The spleen was grossly enlarged (1,400 g.), and had some infarcts. The prostate was enlarged, but not hard; it contained a few small firm masses. The marrow of the lower lumbar vertebrae was leukaemic. In the prostate was a moderately well differentiated carcinoma. The spleen and glands showed typical lymphatic leukaemia.

COMMENT

There is no proof that this patient did not have leukaemia before receiving stilboestrol therapy. The leucocyte count was not performed, but, if normal, lymphatic leukaemia would not have been excluded, as experience shows that when all cases of anaemia are exhaustively investigated the aleukaemic variety is relatively common. The haemoglobin and red-cell count were almost normal at the beginning of therapy; no splenic enlargement was noted, but posterior cervical glands were present. If leukaemia were already present before stilboestrol therapy, progress would at this age be slow, and the cell type was well differentiated.

Thus there are three alternatives: the stilboestrol induced the leukaemia; the stilboestrol accelerated the leukaemia; the stilboestrol did not affect the course of the leukaemia. But a lymphocytic leukaemia of well-differentiated cell type in an elderly person presented as a rapidly progressive and fatal anaemia. Therefore I regard this last alternative as improbable.

Co-operation from Mr. F. I. Evans, Dr. Blair Macaulay, and Dr. N. C. W. Owen is gratefully acknowledged.

D. A. STANLEY, M.D., B.Sc., F.R.I.C.,
Consultant Pathologist, United Liverpool Hospitals.

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