

BLOOD GROUP SEROLOGY—THE FIRST FOUR DECADES (1900–1939)*

by

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SUMMARY

ALTHOUGH BLOOD transfusion had been practised spasmodically since the seventeenth century it was not until the discovery of the blood groups in 1900 that it became a potentially safe procedure, and pre-transfusion compatibility testing could be undertaken.

Blood grouping and transfusion practice until the second world war remained very primitive, however. Some of the techniques in use in the 1920s and 1930s are here described and discussed, and some of the specific laboratory problems which arose are considered in the light of present knowledge. In particular, the mystique which then surrounded blood group serology is explained in terms of the confusion aroused by the existence of different nomenclatures for the ABO groups, and the lack of techniques for demonstration of (what are now known as) IgG antibodies. The reluctance of clinicians to use blood transfusion during this period is explained partly as a consequence of this limited serological understanding.

INTRODUCTION

The concept of blood transfusion is an ancient one, but although attempts at the practice were made at various times during the seventeenth, eighteenth, and nineteenth centuries there was no real success until Blundell's clear demonstration in 1825 that "in performing the operation of transfusion on the human body, the human blood should alone be employed"¹ and not that of any other animal species. Despite this observation, blood transfusion did not achieve any further substantial stimulus until after the publication in 1900² of Landsteiner's observations of what he subsequently called "the unexpected existence of clearly demonstrable differences between the bloods within one animal species".³ Landsteiner defined three different groups, and in 1901 von Decastello and Sturli discovered a fourth.⁴

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¹ J. Blundell, *Researches physiological and pathological*, London, Cox, 1825, p. 92.

² K. Landsteiner, 'Zur Kenntniss der antifermentativen, lytischen und agglutinierenden Wirkungen des Blutserums und der Lymphe', *Zbl. Bakt.*, 1900, 27: 357–362.

³ K. Landsteiner, 'Ueber agglutinationserscheinungen normalen menschlichen blutes', *Wien. klin. Wschr.*, 1901, 14: 1132–1134. (A translation into English was published by A. L. Kappus, *Transfusion*, 1961, 1: 5–8.)

⁴ A. von Decastello and A. Sturli, 'Ueber die Isoagglutinine im Serum gesunder und kranker Menschen', *Munch. med. Wschr.*, 1902, 49: 1090–1095.

PROBLEMS OF NOMENCLATURE

In the early part of the present century communications were less than ideal in the medical and scientific fields. Independently, two other workers—in addition to Landsteiner, and von Decastello and Sturli—demonstrated that human blood could be classified into four groups. Jan Jansky, a Czech, published his work in 1907⁵ in an obscure local journal. Despite the inclusion of a résumé in French,⁶ Jansky's work went largely unnoticed, and in America Moss⁷ published his own (very similar) work in 1910.⁸

In itself this duplication was nothing but a useful confirmation of the new knowledge, but unfortunately inadequacies of communication led to duplication not only of work, but also of the nomenclatures devised to describe the new blood groups.

Landsteiner had designated his three groups by the letters A, B, and C—the latter being that in which “the serum agglutinates the red blood cells of Group A and B but the red blood cells of C are not influenced by the sera of A and B”⁹—while von Decastello and Sturli had not given any particular name to the fourth group which they had discovered.

Jansky had used Roman numerals to identify his four blood groups and Moss did likewise—but whereas (by chance) both Jansky and Moss had used II for Landsteiner's A, and III for his B, they differed over the usage of I and IV, Jansky referring to Landsteiner's C as I, and to the group of von Decastello and Sturli as IV, while Moss reversed this, calling Landsteiner's C group IV, and von Decastello and Sturli's group, I.

The possibilities of confusion were endless—and potentially lethal in transfusion practice. In Britain, France, and parts of the United States the nomenclature of Moss was preferred—probably because of the relative accessibility of his paper, written in English—while elsewhere (including other parts of the U.S.A.) Jansky's system was in use. In an attempt to rationalize the situation special committees were appointed by the American Association of Immunologists, the Society of American Bacteriologists, and the Association of Pathologists and Bacteriologists. They made a joint recommendation¹⁰ in 1921 that, “on the basis of priority . . . the Jansky classification be adopted”. Unfortunately, Moss's system was so generally used in Europe that the net effect was the establishment of different nomenclatures on each side of the Atlantic, and even in the United States it was said shortly afterwards that “The practically universal use of the Moss classification at that time was completely and purposely cas-

⁵ J. Jansky, ‘Haematologické studie u. psychotiku’, *Sborn. Klinický*, 1907, 8: 85–139.

⁶ *Ibid.*, pp. 131–133.

⁷ W. L. Moss, ‘Studies on isoagglutinins and isohemolysins’, *Bull. Johns Hopk. Hosp.*, 1910, 21: 63–70.

⁸ Moss did discover Jansky's paper while his own was in the press, and acknowledged it—and Jansky's precedence—in a footnote.

⁹ Landsteiner, *op. cit.*, note 3 above (trans.), p. 7.

¹⁰ Report, ‘Isohemagglutination. Recommendation that the Jansky classification be adopted for universal use’, *J. Amer. med. Assn.*, 1921, 76: 130.

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aside. Therefore in place of bringing order out of chaos, chaos was increased in the larger cities".¹¹

In 1922 Landsteiner started working at the Rockefeller Institute for Medical Research in New York, and it was as a member of a committee of the National Research Council concerned with blood grouping that he "suggested the substitution of the well known letters O, A, B and AB for the Jansky numbers I, II, III and IV and the Moss numbers IV, II, III and I".¹² Landsteiner's suggestion was, almost immediately, taken up widely in both Europe and America and is that which is in use today (Table I). Despite this clarification, Moss's nomenclature was in not uncommon use—even if only as an addition to the "International" system—until well into the 1950s. The present author clearly recalls the use of such terms as "O, IV" and "A, II" in the period 1947–57—despite the obvious risk of confusion of the latter with the sub-group A₂.

TABLE I BLOOD GROUP NOMENCLATURES, 1900–1927¹³

Original Landsteiner (1901)	Jansky (1907)	Moss (1910)	"International" (1927)
C	I	IV	O
A	II	II	A
B	III	III	B
Von Decastello and Sturli	IV	I	AB

Quite apart from the risks of confusion inherent in the simultaneous existence of three nomenclatures for the red cell types, the widespread use of numerical systems combined with the verbal jargon of early blood group serology to make this complex new subject more obscure to the non-expert than it need have been. Antibodies were referred to (quite reasonably) as iso-agglutinins, and red cell receptors as agglutinogens—that is, the latter were said to possess "agglutinophilic capacity". However, the lack of a vocabulary which adequately expressed what today would be called "antithetical antigen/antibody relationships" led to such descriptions of the reactions of blood groups as that given by Keynes, in his classical monograph published in 1922.¹⁴ He said:

The corpuscles of Group I are agglutinated by the sera of II, III, IV. The corpuscles of Group II are agglutinated by the sera of III, IV. The corpuscles of Group III are agglutinated by the sera of II, IV. The corpuscles of Group IV are not agglutinated by any of the other groups.

On the other hand:

The serum of Group I agglutinates no other corpuscles. The serum of Group II agglutinates the corpuscles of Groups I, III. The serum of Group III agglutinates the corpuscles of Groups I, II. The serum of Group IV agglutinates the corpuscles of Groups I, II, III.

¹¹ J. A. Kennedy, 'Blood group classifications used in hospitals in the United States and Canada', *ibid.*, 1929, 92: 610–615.

¹² 'Current comments', *ibid.*, 1927, 88: 421–422.

¹³ Taken from N.S.R. Maluf, 'History of blood transfusion', *J. Hist. Med.*, 1954, 9: 90.

¹⁴ G. Keynes, *Blood transfusion*, London, Frowde, 1922, p. 71.

Such a statement is only just more easily digested when produced in tabular form (Table II).

TABLE II TABLE OF THE BLOOD GROUP REACTIONS¹⁴

		Serum			
		I	II	III	IV
	I	—	+	+	+
Corpuscles	II	—	—	+	+
	III	—	+	—	+
	IV	—	—	—	—

As early as 1910 Von Dungern and Hirszfild¹⁵ had named the two isoagglutinins described by Landsteiner with the Greek letters α (alpha) and β (beta)—referring respectively to what we today call anti-A and anti-B (that is, the agglutinins of Moss and Jansky's groups III and II, respectively). Unfortunately this usage did not become popular until the second world war and a large number of terms were coined to describe the ABO antibodies, none of which did anything to aid wider understanding of the serologist's jargon. Indeed, to make confusion worse, some workers produced their own terms for Landsteiner's A and B, referring to these as *b* and *a* respectively—meaning that a group A blood had receptors for the agglutinins of group B, and vice versa. Guthrie and Huck¹⁶ used this system (without much explanation) in a paper published in 1923, and their terminology is shown alongside the "International" nomenclature in Table III. In this scheme the agglutinins of A and B are named directly after the group in which they are found—but with a reversal of the group O and AB nomenclatures similar to that in the numerical systems of Moss and Jansky.

TABLE III VARIATIONS OF THE ABO NOMENCLATURE FOR RED CELL "AGGLUTINOGENS" AND SERUM "AGGLUTININS"

"International"		Guthrie and Huck ¹⁶	
Red cells	Serum	Red cells	Serum
AB	—	ab	O
A	Anti-B (beta)	b	A
B	Anti-A (alpha)	a	B
O	Anti-A + B (alpha, beta)	o	AB

¹⁵ E. von Dungern and L. Hirszfild, 'Ueber Vererbung-gruppen spezifischer Strukturen des Blutes', *Z. Immun. Forsch. Orig.*, 1910, 6: 284–292. (A translation into English was published by G. P. Pohlmann, *Transfusion*, 1962, 2: 70–74.)

¹⁶ C. G. Guthrie and J. G. Huck, 'On the existence of more than four isoagglutinin groups in human blood', *Bull. Johns Hopk. Hosp.*, 1923, 34: 37–48.

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Not until time, common sense, and an improved degree of international co-operation produced agreement on a single nomenclature for both antigens and antibodies could there be any hope of blood group serology appearing as anything but a series of mystical incantations in the eyes of the clinicians who were expected to heed its warnings. In 1942 Bernheim recalled that in Boston in 1910 “not once was it even suggested that I had better wait for blood tests, and I did transfusions in every hospital in the city and many in distant communities”.¹⁷ Such disregard of blood grouping was not easily overcome while its language was so esoteric and obscure.

TECHNIQUES OF BLOOD GROUPING

Blood grouping techniques are theoretically very simple, requiring only that sera and cells are brought together in optimal ratios and allowed to react for an adequate period of time before being examined for evidence of a reaction between them. In practice, as any modern serologist will confirm, the procedure is fraught with problems of chemistry, of the ability of the serologist to interpret what he sees, and of ensuring the potency and specificity of one's reagents. Until comparatively recently many of these problems were resolved only by an empirical approach—and it took a long time for many of the difficulties to be eliminated.

Landsteiner described his own grouping technique very simply in 1901. He said: “Equal amounts of serum and of 5 per cent red blood cell suspension in 0.6 per cent sodium chloride were mixed and observed in a hanging drop preparation or in the test tube”.¹⁸ The reason for using 0.6 per cent saline is not clear, but the use of a hanging drop preparation was a clear adaptation of the bacteriological laboratory techniques which were to have a considerable influence in the early development of blood grouping.

In 1916 Brem¹⁹ introduced an elaborate method of testing in which he mixed two “Platinum loopfuls” of serum and one of a five per cent suspension of cells on a coverslip, which was inverted over a microscope cavity slide and sealed with petroleum jelly. The presence or absence of agglutination was observed microscopically after fifteen minutes. Apart from the niceties of technique, one of the objections offered to this method by Keynes²⁰ in 1922 was that of distinguishing true agglutination from rouleaux—although one would have expected the use of saline suspended cells (rather than whole blood) to have minimized the formation of rouleaux. Nevertheless, the use of hanging drop preparations was still being strongly recommended as late as 1931.²¹

An even more elaborate bacteriological procedure was proposed in 1924 by Learmonth,²² in Glasgow—although the intention here was to maintain the sterility of his anti-sera. Learmonth's sera were “obtained under aseptic precautions from known

¹⁷ B. Bernheim, *Adventures in blood transfusion*, New York, Smith & Durrell, 1942.

¹⁸ Landsteiner, 1901, op. cit., note 3 above (trans.), p. 6.

¹⁹ W. V. Brem, ‘Blood transfusion, with special reference to group tests’, *J. Amer. med. Assn.*, 1916, 67: 190–193.

²⁰ Keynes, op. cit., note 14 above, p. 103.

²¹ E. J. A. Kolmer and F. Boerner, *Approved laboratory technic*, New York, Appleton-Century, 1931, p. 449.

²² J. R. Learmonth, ‘Human blood-grouping; a review of its present position and applications’, *Glasg. med. J.*, 1924, 101: 116–134.

individuals in Groups II and III, and stored in the dark in the ice-chest in sterile bottles with closely-fitting ground-glass stoppers". The technique used for grouping was to mix the reagents on a plain microscope slide, the sera being removed from their bottles with a sterile loop of platinum wire, 4 mm. in diameter, which was flamed and cooled before each insertion. Whole blood from a finger-prick was then added to each slide by means of the freshly flamed loop. Agglutination was observed, usually "within a minute or two and not longer than ten minutes". Longer incubation was said to lead to false positive reactions, due to drying of the test and the "consequently increased salt concentration of the mixture". Using this method Learmonth claimed that his "test sera . . . retain their activity for at least four months".²³ Even as recently as the 1950s an elderly blood group serologist, who had started his laboratory career in bacteriology, could be found in an Australian laboratory employing a similar technique.²⁴

That rouleaux formation was (not surprisingly) a problem to Learmonth, as it had been to Brem, was clear for he noted that "It cannot be too strongly insisted that the use of the microscope to determine agglutination may introduce a serious source of error, especially if the observer has little experience of the test, since *rouleaux* formation is thus liable to be mistaken for agglutination".²⁵

The mistrust of microscopical reading methods expressed by Learmonth has been one of the more long-standing misapprehensions in blood group serology. In 1939 Riddell said that "The use of the microscope in inexperienced hands is a frequent cause of false positive results, as *rouleaux* formation is more obvious and is easily mistaken for true agglutination",²⁶ while as recently as 1974 an American author wrote ". . . do not use a microscope for most agglutination processes . . . [as] false positive readings may be reported".²⁷

Riddell, paradoxically, was both right and wrong. Rouleaux is more obvious under the microscope, but it is for that very reason that it is less easily mistaken for agglutination when examined microscopically than macroscopically. Perhaps the basic false premise—which is still common today—was that blood grouping can, or should, be performed at all by inexperienced workers.

Slide, or tile, techniques of blood grouping have always been popular. Writing in 1939 Riddell suggested that "A saucer or teacup turned upside down will serve very well in an emergency",²⁸ although opal glass or porcelain tiles were more common. When using microscope slides the disadvantage of rapid drying of the tests was generally overcome by placing a coverslip over the preparation and then reading up to fifteen minutes later²⁹ although, as Riddell pointed out, this meant that "the free migration of the red cells may be interfered with".³⁰ It is surprising that the earliest reference which has been traced to the use of any form of moist chamber to overcome

²³ *Ibid.*, p. 121.

²⁴ H.B.M. Lewis, 1978. Personal communication.

²⁵ Learmonth, *op. cit.*, note 22 above, p. 122.

²⁶ V. H. Riddell, *Blood transfusion*, Oxford University Press, 1939, p. 36.

²⁷ B. A. Myhre, *Quality control in blood banking*, New York, Wiley, 1974, p. 110.

²⁸ Riddell, *op. cit.*, note 26 above, p. 24.

²⁹ Kolmer and Boerner, *op. cit.*, note 21 above, p. 450.

³⁰ Riddell, *op. cit.*, note 26 above, p. 24.

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drying is in an American book published in 1942, where it was noted that “If no agglutination is seen, the slide is placed under a Petri dish cover with a piece of wet cotton and a final reading is made after fifteen minutes”.³¹ Given a suitable form of moist chamber, tile techniques are still valuable for a number of grouping purposes today.

It is the tube technique—first mentioned by Landsteiner in 1901, and refined by Moss in 1910—which has become the classical manual technique in blood grouping, however. With the exception of the volumes of reagents used, and the temperature of incubation adopted, there have been few modifications to this technique for the last seventy-eight years.

SOME SEROLOGICAL PROBLEMS

The three great deficiencies in blood grouping during the period up to 1940 were: (1) lack of knowledge of the complexity of blood group immunology; (2) lack of a technique for demonstrating antibodies of the IgG type, and (3) lack of sufficient experience to enable the development of techniques to resolve the first two deficiencies. These three problems formed a closed system from which it was difficult to break; and that system itself could not break free from the reputation of blood transfusion as a clinically dangerous procedure, which itself inhibited the acquisition of the experience which alone could break the vicious circle.

That the existence of unknown factors in the blood was early appreciated is clear from Keynes' statement in 1922 that: “the view is gaining ground that there may be some ‘overlapping’ of groups, that is to say, a serum may contain agglutinins which give a gross reaction with the corpuscles of one group and a reaction with another group so slight that it can be detected only with difficulty, or alternatively the recipient's corpuscles may give a definite and limited group reaction, while his serum may cause some agglutination in the blood of a theoretically compatible group”.³² In 1921 Unger³³ had referred to these properties as “‘chief’ or ‘major’” and “‘para’ or ‘minor’” agglutinins, and said that their presence made the direct testing of recipients' blood with that of donors advisable prior to every transfusion—although possibly the first recorded reference to the need for pre-transfusion direct compatibility tests as well as blood grouping had been made ten years earlier in 1911, by Ottenberg³⁴ of the Laboratory of Biological Chemistry at Columbia University.

The problems foreseen by Keynes, Unger, and others were, of course, the presence of clinically significant antibodies outwith the ABO system, and occurring in the blood of either recipients or donors. The identity of such antibodies could not readily be ascertained in the 1920s and 1930s and thus some form of direct matching was clearly called for.

A typical example of the problems experienced by early serologists was given in the

³¹ R. A. Kilduffe and M. DeBakey, *The blood bank and the technique and therapeutics of transfusions*, St. Louis, Mosby, 1942, p. 172.

³² Keynes, *op. cit.*, note 14 above, pp. 72–73.

³³ L. J. Unger, ‘Precautions necessary in the selection of a donor for blood transfusion’, *J. Amer. med. Assn.*, 1921, 76: 9–11.

³⁴ R. Ottenberg, ‘Studies on isoagglutination. I. Transfusion and the question of intravascular agglutination’, *J. exp. Med.*, 1911, 13: 425–438.

paper of Coca and Klein published in 1923, in which they reported 'A hitherto undescribed pair of isoagglutination elements in human blood'.³⁵ As a result of some absorption experiments these workers had "discovered" an apparently new antithetical antigen/antibody in which the antigen—which they called X—was present in eighty per cent of group A and AB bloods.

Looked at with hindsight it is almost certain that Coca and Klein had re-discovered the sub-division of the A antigen into A₁ and A₂, by an antibody with the specificity of anti-A₁. Von Dungern and Hirszfeld had first described this distinction in 1911³⁶ but, in view of the problems of language and the confusion engendered by the multiplicity of nomenclatures then current, it is not surprising that this was lost sight of. Our present understanding of the A₁/A₂ division dates only from about 1930, indeed, following the work of Friedenreich and his colleagues.³⁷

The absorption technique used by Coca and Klein to separate their two antibodies (probably anti-A and anti-A₁) consisted of adding one drop of washed packed cells to 1 cm³ of serum every two minutes, up to a total of eight drops. It was clearly inadequate in the light of modern knowledge, and explains the finding of the "new" antibody (probably anti-A₁ but which they called "agglutinin x") in only seventy-two per cent of the group O and B sera which they examined. The remaining twenty-eight per cent would have been insufficiently absorbed so that only anti-A was demonstrable.

It was not only the presence of agglutinins against unknown antigens which posed problems. In 1923 Guthrie and Huck³⁸ described a patient whose blood grouped as B, but who had no anti-A in her serum. In a lengthy article they described their studies upon the patient—an eighteen-year-old girl suffering from sickle cell disease—and her family, in which another three examples of the phenomenon were found (Fig. 1). From the apparently hereditary nature of the discrepancy it seems reasonable to assume that it was a red-cell rather than a serum phenomenon, and one might hazard a guess that this was the first recorded example of the cell type A_x, occurring in the combination A_xB. If this is so, the family pedigree could be expressed as in Fig. 2. The case pre-dates by twelve years the first example of A_x hitherto described in the literature.³⁹

Blood group anomalies frequently run together and this particular case was complicated by the presence of an irregular antibody in the serum of the propositus (C.T.Jnr). Guthrie and Huck were restricted in the extent to which they could investigate this, but it seems that the antibody reacted strongly with the cells of fourteen

³⁵ A. F. Coca and H. Klein, 'A hitherto undescribed pair of isoagglutination elements in human beings', *J. Immunol.*, 1923, 8: 477-485.

³⁶ E. von Dungern and L. Hirszfeld, 'Über gruppenspezifische Strukturen des Blutes. III', *Z. Immun. Forsch.*, 1911, 8: 526-562. (A translation into English has been published by the Blood Transfusion Division, U.S. Army Medical Research Laboratory, Fort Knox, Kentucky 40121, U.S.A.)

³⁷ O. Thomsen, V. Friedenreich, and E. Worsane, 'Über die Möglichkeit der Existenz zweier neuer Blutgruppen; auch ein Beitrag zur Beleuchtung sogenannter Untergruppen', *Acta path. microbiol. Scand.*, 1930, 7: 157-190; V. Friedenreich and A. Zacko, 'Die Differentialdiagnose zwischen den "Unter"-gruppen A₁ und A₂', *Z. Rassenphysiol.*, 1931, 4: 164-191; V. Friedenreich, 'Ueber die Serologie der Untergruppen A₁ und A₂', *Z. Immun. Forsch.*, 1931, 71: 283-313.

³⁸ Guthrie and Huck, op. cit., note 16 above.

³⁹ W. Fischer and F. Hahn, 'Ueber auffallende Schwarde der gruppenspezifischen Reaktionsfähigkeit bei einem Erwachsenen', *Z. Immun. Forsch.*, 1935, 84: 177-188. (A translation into English has been published. See note 36 above.)

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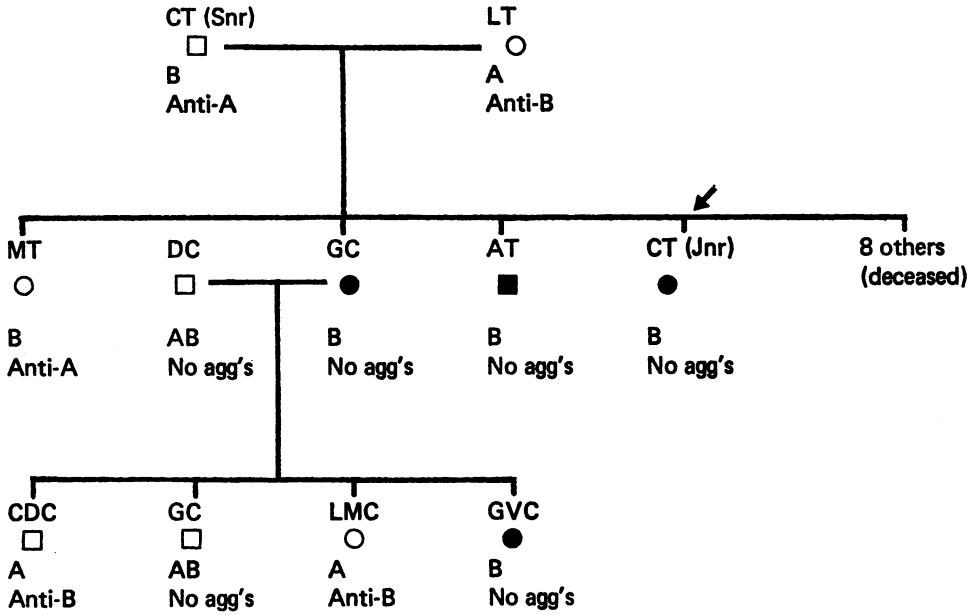


Figure 1. Family pedigree of Patient C T (Jnr), showing blood groups determined by Guthrie and Huck.³⁰

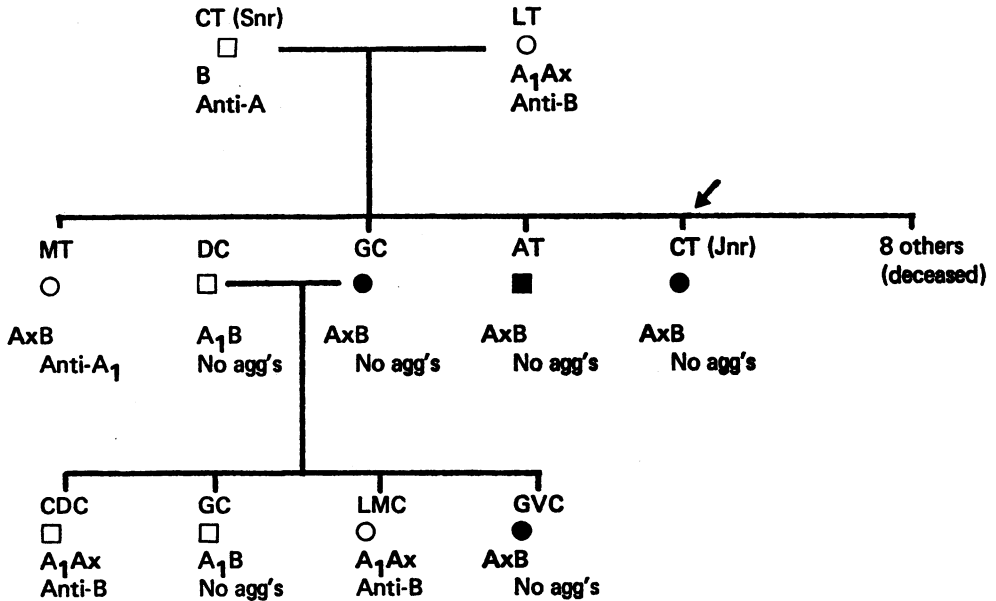


Figure 2. Family pedigree of Patient C T (Jnr), showing possible explanation for unusual grouping reactions.

other persons (all group A) but not with 176 others (comprising about sixty-nine group A, twenty-eight group B, fifteen group AB, and sixty-four group O⁴⁰), that is, it revealed an antigen present in 7.4 per cent of the bloods tested. Absorption experiments of a surprising elegance for the period showed that the antibody was highly specific, and apparently unrelated to the ABO groups. It is not recorded whether or not the patient had ever been transfused, and at this interval in time it is not possible to identify the antibody with any degree of confidence. It seems likely from the evidence, however, that this was an example of anti-Lu^a. (Other possibilities include the very rare alternatives of an apparently naturally-occurring IgM anti-K, or maybe the equally rare anti-M'.) The first recorded anti-Lu^a in modern times was not reported until 1945.⁴¹

Such "problem" cases, explicable in the light of modern knowledge but utterly baffling to contemporary workers, were not uncommon in the literature of the 1920s and 1930s.

DIRECT MATCHING

Mostly, techniques recommended for the purpose of direct matching prior to transfusion were variants of those used for ABO grouping,⁴² but two special techniques stand out from the rest.

In 1923 Tzanck,⁴³ in Paris, employed an unusual *in vivo* test in which 0.5 cm³ of the recipient's blood was mixed with the same amount of the blood of the prospective donor and the mixture injected into the heart of a guinea-pig. The animal was said to die almost at once if the bloods were incompatible. The rather dry comment of one contemporary Scottish surgeon was that "This procedure appears to be unnecessary".⁴⁴

The concept of a "biological test" was later developed in detail by Franz Oehlecker of Hamburg, however. In 1933 Oehlecker published a book⁴⁵ in which he described his method. Increasing volumes of 5 cm³, 10 cm³, and 20 cm³ of donor blood were injected into the patient at two-minute intervals. Any haemolytic reactions were said to occur in the first one to two minutes after the injection, and late reactions after a "smooth" transfusion were said to be not haemolytic. In Oehlecker's series of fifty haemolytic reactions detected by his "biological test", there was "only" one death—and that of a patient already moribund.

Thankfully, such drastic measures were not widely adopted and the procedure officially recommended by the Scottish National Blood Transfusion Association as late as 1940 was much more typical of the three decades prior to its publication. These instructions said: "To match the bloods make a suspension of donor's cells . . . then add one drop of this suspension to two drops of serum or haemolysed plasma on a

⁴⁰ Of the 190 cells tested, only sixty-one were of known ABO groups (forty, A; fourteen, B; seven AB). The remainder are calculated as expected figures, based upon known distributions.

⁴¹ S. Callender, R. R. Race, and Z. V. Paykoc, 'Hypersensitivity to transfused blood', *Br. med. J.*, 1945, ii: 83.

⁴² E.g. Kolmer and Boerner, *op. cit.*, note 21 above, p. 448–451.

⁴³ A. Tzanck, 'Les trois grandes variétés de transfusion sanguine', *Paris med.*, 1922, 45: 249–251.

⁴⁴ Learmonth, *op. cit.*, note 22 above, p. 129.

⁴⁵ F. Oehlecker, *Die Bluttransfusion*, Berlin, Urban & Schwarzenberg, 1933.

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slide or watch-glass or porcelain plate. Mix the drops thoroughly, cover with a saucer or Petri dish to prevent evaporation, and keep in a warm place, *e.g.* near an electric lamp. . . . Agglutination is usually visible within two minutes, but ten minutes should be allowed to elapse before compatibility is assumed".⁴⁶

Despite the large amount of serological work which was carried out during the 1920s and 1930s the unpalatable fact was that people still suffered, and sometimes died of, haemolytic reactions following transfusion of blood which was not only homologous, but apparently compatible, with that of the patient. The cause of many of these reactions can today be seen to have been antibodies of the IgG class. Compatibility testing by simple exposure of whole blood—or sometimes cell suspensions—to the recipient's serum for five minutes or so at room temperature would clearly be incapable of demonstrating antibodies now recognized as forming part of, for example, the Rh, Kell, Duffy, or Kidd systems—most of which are potentially lethal *in vivo*. It was not until 1944 that Race⁴⁷ in Britain, and Wiener⁴⁸ in the U.S.A., more or less simultaneously described the "incomplete" antibodies which were the cause of many hitherto unexplained causes of *in vivo* blood group incompatibility, and triggered off the explosion of techniques in blood grouping which has led to our present extensive knowledge of serology.

CONCLUSIONS

During the period prior to the second world war blood grouping was widely performed, but it was severely limited in what could be demonstrated. Primitive techniques and poor international communications undoubtedly played their parts in the situation, but it is submitted that the over-riding factor which stultified development in this field was the lack of a uniform and universally agreed nomenclature for the ABO blood groups.

One has only to read papers such as those by Guthrie and Huck,⁴⁹ and Coca and Klein,⁵⁰ which describe actual practical problems, in order to see the near-impossibility of the authors expressing their results in a manner which was both coherent and capable of ready interpretation. The two pairs of American authors mentioned above wrote within a few months of one another, one using Moss's nomenclature and the other Jansky's. Cross-reference between such papers can be incredibly difficult, even to a modern serologist provided with a key and with the benefit of an extra fifty years' knowledge and serological experience to draw upon: to contemporary workers it would have been daunting indeed.

Serologically, the three decades before 1940 were a period of considerable confusion and misunderstanding, which saw very little real advance in knowledge of the blood groups. It is true that, in a brilliant series of experiments Landsteiner and Levine

⁴⁶ C. F. W. Illingworth, *Transfusion of stored blood in the treatment of war casualties*, Edinburgh, Scottish National Blood Transfusion Association, 1940, p. 7.

⁴⁷ R. R. Race, 'An "incomplete" antibody in human serum', *Nature, Lond.*, 1944, 153: 771–772.

⁴⁸ A. S. Wiener, 'A new test (blocking test) for Rh sensitization', *Proc. Soc. exp. Biol., N. Y.*, 1944, 56: 173–176.

⁴⁹ Guthrie and Huck, *op. cit.*, note 16 above.

⁵⁰ Coca and Klein, *op. cit.*, note 35 above.

demonstrated the existence of the M, N and P antigens in 1927–28,⁵¹ but the antibodies concerned were hetero-immune products of the deliberate immunization of rabbits with human red cells, and they were neither found in human sera until some years later (anti-M in 1933;⁵² anti-N in 1937;⁵³ anti-P—now called anti-P₁—in 1930⁵⁴), nor were they of real clinical significance.

Serology was stultified by its own limited techniques and the lack of a uniform nomenclature, and as a result clinicians remained wary of a procedure which could still produce severe, even fatal, reactions despite the serologist's efforts. Conversely, the wary approach of clinicians to transfusion deprived the serologist of much of the stimulus needed to force a resolution of his problems, as well as restricting the clinical material needed for the discovery of new antigen/antibody reactions.

Not until the “breakthrough” of the elucidation of the Rh blood groups in 1940⁵⁵ coincided with the stimulus of a world war—with its urgent needs for the treatment of massive numbers of casualties—did blood group serology break free of the vicious circle in which it found itself, and become the advanced, and practically orientated, subject which it is today.⁵⁶

⁵¹ K. Landsteiner and P. Levine, ‘A new agglutinable factor differentiating individual human bloods’, *Proc. Soc. exp. Biol., N.Y.*, 1927, 24: 600–602; ‘Further observations on individual differences of human blood’, *ibid.*, pp. 941–942; ‘On individual differences in human blood’, *J. exp. Med.*, 1928, 47: 757–775.

⁵² E. Wolff and B. Jonsson, ‘Studien über die untergruppen A₁ und A₂ mit besonderer berucksichtigung der paternitätsuntersuchungen’, *Dt. Zt. gerichtl. Med.*, 1933, 22: 65–85.

⁵³ S. Iseki, T. Fukao, and J. Suzuki, ‘On the Anti-N agglutinin found in normal human serum’, *Hanzaigaku-Zasshi*, 1937, 11: 245–242 (in Japanese).

⁵⁴ K. Landsteiner and P. Levine, ‘On the inheritance and racial distribution of agglutinable properties of human blood’, *J. Immunol.*, 1930, 18: 87–94.

⁵⁵ For a valuable collection of facsimile reprints of the most important papers dealing with this, see C. A. Clarke (ed.), *Rhesus haemolytic disease*, London, Medical and Technical Publishing Co., 1975, pp. 4–102.

⁵⁶ In 1977 at least 398 blood group antigens were known. See P. Issitt and C. H. Issitt, *Applied blood group serology*, Oxnard (Calif.), Spectra, 1977, chap. 28.