although simpler to perform, has given too many equivocal results to be satisfactory. It has also given more false positives and false negatives than the quininium test. It is true that we checked it against the much more stringent augmented histamine test, but this would not explain the false positives.

There is evidence that if tubeless gastric analysis is repeated in cases in which an equivocal result has been obtained, a definitive result may be obtained in the second test (Flood *et al.*, 1953; Galambos and Kirsner, 1955; Sievers and Gieselmann, 1956). It has also been suggested that histamine should replace caffeine benzoate as the gastric stimulant in tubeless gastric analysis. Indeed, Galambos and Kirsner (1955) found only one false positive and one false negative among 104 patients using betazole hydrochloride ("histalog") instead of caffeine in the azure A test. We are, however, interested in a simple test for achlorhydria which can be performed at home by the patient, and the introduction of parenteral histamine would take tubeless gastric analysis out of the category of screening tests.

The exchange-resin methods have no value as quantitative tests of gastric acidity, as the amounts of cation displaced from the resin, absorbed from the intestine, and excreted in the urine are not directly proportional to the amount of hydrochloric acid secreted by the stomach (Segal *et al.*, 1955; Galambos and Kirsner, 1955; Sievers and Gieselmann, 1956). The resins are therefore of little use in the study of hyperchlorhydria and peptic ulceration. Their role is in the detection of diseases associated with achlorhydria, such as pernicious anaemia and gastric carcinoma.

Summary

The literature on tubeless gastric analysis is briefly reviewed and the circumstances under which such tests are likely to be unreliable are mentioned.

The quininium resin test was compared with the single-dose histamine test meal in the recognition of achlorhydria; agreement was satisfactory.

The azure A resin was compared with the augmented histamine test. The results were unsatisfactory, as there was too high a proportion of equivocal results. In addition, false positive and false negatives were more frequent than in the quininium test.

The quininium test is a valuable method of screening patients for achlorhydria, but the azure A method, although simpler to perform, cannot be recommended.

We thank Dr. Sheila Callender for her help and Mrs. Allison Jones and Miss Sandra Reynolds for technical assistance. We are grateful to Messrs. E. R. Squibb and Sons, Ltd., for putting the exchange resins at our disposal.

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A NEW EXAMPLE OF THE Rh ANTIBODY, ANTI-C*

BY

GERTRUDE PLAUT, M.D.

P. B. BOOTH, M.R.C.S., L.R.C.P.

North London Blood Transfusion Centre

CAROLYN M. GILES, B.Sc.

AND

A. E. MOURANT, D.M., M.R.C.P.

Blood Group Reference Laboratory

This paper records the finding and investigation of the rare antibody, anti-C*, in the serum of a patient and the investigation of a number of persons who were thereby shown to carry the corresponding antigen.

Present Investigation

The patient, Mrs. T., was born in 1911; she had two children, born in 1938 and 1940. The first of these died at the age of 1 week from a cause which it is now almost impossible to ascertain owing to the lapse of time. There is no record of any other pregnancy, of any transfusion, or of any injection of blood prior to the test in which the abnormal antibody was discovered.

In January, 1957, the patient was found to have an adenocarcinoma of the caecum, and was admitted for operation to the Hospital of St. John and St. Elizabeth, London. As it was anticipated that a blood transfusion would be needed she was grouped, and found to be group O, Rh(D) positive; two bottles of group O, Rh-positive packed cells (each from two donors) were then tested for compatibility with her serum. One bottle was found to be incompatible both by saline and by albumin tests. Further tests with her serum showed no agglutination of 13 other donor samples examined, or of seven samples from the transfusion centre staff, selected as covering a wide range of antigens. In all, blood from 11 donors was transfused uneventfully. The two donors who had contributed to the incompatible bottle of packed cells were recalled for further investigation.

At the Blood Group Reference Laboratory the patient's serum was tested against red cells from both these donors and from a selected panel of 22 other bloods, including known examples of the antigens W_r^a , M_i^a , V^w , and one other "family" antigen, so far unpublished. Tests were carried out in saline and albumin at 37°, 18°, and 4° C., and by the indirect anti-human-globulin method; the serum was also tested by Löw's method with cystein-activated papain, and against trypsin-treated cells. All the results were negative except in the case of the blood from one of the two contributors to the incompatible bottle, Mrs. W., which reacted in all the tests except at 4° C. in saline.

The red cells of this donor's father and sister, but not those of her mother, were also found to be agglutinated by the serum; those of the patient herself, her husband, and her surviving son were unagglutinated.

The cells of the donor, Mrs. W., were grouped as fully as possible with available standard sera. They were tested also with reagents for the antigens W_r^a , M_i^a , V^w , V , $Diego$, C^x , E^w , f , Be^a , R_m , U , Vel , and T_j^a , and for two other rare "family" antigens at present under investigation, and were found to react only with anti- C^x , anti- U , anti- Vel , and anti- T_j^a , the last three of which react with nearly all known bloods. This result strongly suggested that the patient's antibody was anti- C^x . Through the kindness of Dr. F. Stratton, who had supplied the known anti- C^x used in the tests, we received a sample of known C^x -positive

blood. This was found to react with the patient's serum. The two relatives of Mrs. W. whose cells had been found to give positive reactions with the patient's serum were also found to be C^x-positive; as described below, the red cells of an unrelated person whose cells had previously given anomalous results with anti-C sera were also tested, and they too were found to react both with the original anti-C^x and with the patient's serum.

It was now abundantly clear that the patient's serum contained anti-C^x, and that the donor Mrs. W., her father, and her sister carried the antigen C^x.

On the assumption, later confirmed, that the anti-C serum used in the full grouping of all the bloods reacted also with the C^x antigen, it was possible to deduce the C-C^x-c genotypes and, with a high degree of probability, the full Rh genotypes of the donor and members of her family. These are shown, together with the rest of their blood groups and those of the patient and her husband and son, in Table I. Three sisters and one brother of the patient were also fully tested: all proved to be C^x-negative.

A panel of 25 different anti-C sera was tested against blood specimens containing combinations of the antigens C, C^w, C^x, and c, as well as against two bloods known to contain C variants. One of these was at once found to be C^x (with c), while the other remains unclassified (see Tables II and III). All the C^xc bloods gave nearly identical patterns of positive and negative results, which differed from those given by C^wc and by the other variant; the few differences between the C^xc bloods are probably due to the greater age of some of the samples. Some anti-C sera were shown to contain both anti-C^w and anti-C^x, others to contain one or

the other, and some to contain neither. Four pure anti-C^w sera, supposedly free from other antibodies, were tested against a variety of cells containing the C^x antigen, and were thereby shown to be free from anti-C^x. Four anti-c sera were tested against C^xC cells and also shown to be free from anti-C^x (see Table III).

Titration of one blood of genotype C^xC and one of genotype C^xc against anti-C^x serum showed that the C^x antigen reacts equally well in the presence of C or of c; it is not detectably depressed by the presence of C.

In order to determine the optimal conditions for agglutination by the patient's serum, it was titrated against C^x-positive cells at 37° and 18° C. in saline and in albumin, and at 37° C. with papainized cells. In all cases titres were highest at 37° C., the titres being 2 in saline, 16 in albumin, and 32 with papainized cells. The indirect anti-human-globulin technique gave relatively poor results.

Discussion

Stratton and Renton (1954) showed very clearly that the antigen C^x was the product of a gene belonging to the CC^wc set of alleles of the Rh series. The genetics of the family of Mrs. W. support this conclusion, as do our observations of the reactions given by the various anti-C sera with C^x-positive cells.

As it was assumed that the antibody arose spontaneously and not as a result of immunization, the sera of three brothers and one sister of the patient (all, as mentioned above, C^x-negative) were tested for anti-C^x, with uniformly negative results.

TABLE I.—Blood Groups of Patient, Donor, and Relatives of both

	ABO	Rh	MN	S	P	Lu ^a	K	Le ^a	Le ^b	Fy ^a
Mrs. T. (patient)	O CcDee	CDe/cde	MN	—	—	+	—	—	?	—
Mr. T. (husband)	A ₂ B CcDee	CDe/cde	MN	—	—	—	—	—	+	—
Mr. T. (son)	B Ccdee	cde/cde	N	—	—	—	—	+	—	+
Mrs. W. (donor)*	O C ^x CDeFF	C ^x DeF/CDeF	M	+	+++	+	—	—	+	+
Mr. C. (father)	A ₁ C ^x cDee	C ^x De/cde	MN	+	+++	+	—	—	+	+
Mrs. C. (mother)	O CCDee	CDe/CDe	M	—	++	—	—	+	—	—
Miss C. (sister)*	O C ^x CDeFF	C ^x DeF/CDeF	MN	—	+++	+	—	—	+	+

* Samples from these two persons only were tested with anti-f.

TABLE II.—Reactions of Red Cells of Various C Locus Genotypes with a Panel of Saline Agglutinating Anti-C Sera*
(The scoring of reactions is that recommended in M.R.C. Memorandum No. 27)

Cells:	Ain	Bas	Hig	Joh	Lit	Log	MgB	MgN	Pea	Scr	Shr	Shw	Sh452	Sh549	Sh655	Sh800	Wat	Wel
†Cc	+++	+++	+++	++	+++	+++	+++	+++	++	+++	+	+++	+++	+++	+++	(+)	+++	+++
cc	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
E.B. C ^w c	—	+++	+++	+	+++	+	—	—	—	+++	—	+	+	w	+++	—	—	+++
Gam. C ^w c	—	+++	+++	w	w	w	++	?	—	+++	w	+++	w	+++	+++	—	—	+++
Miss Ch. C ^x C	+++	+++	+++	+	+++	+++	+++	+++	++	+++	+++	+++	+++	+++	+++	+	+++	+++
Mr. Ch. C ^x c	—	w	—	—	+++	w	—	w	—	+++	+++	+++	—	+++	+++	—	—	+++
†Miss H. C ^x c	—	w	—	—	+++	—	—	w	—	+++	+++	+++	—	+++	+++	—	—	+++
Miss E. C ^x c	—	—	—	—	+++	—	—	w	—	+++	+++	+++	—	+++	+++	—	—	+++
Mrs. W. C ^x C	++	+++	+++	++	+++	+++	—	—	—	+++	+++	+++	++	++	+++	—	(+)	+++

* Mostly containing "incomplete" anti-D as well. † The Cc control was of phenotype CcDee in all cases but two (Ain and Wat), for which it was Ccdee. ‡ Frozen cell sample only.

TABLE III.—Reactions of Red Cells of Various C Locus Genotypes with a Panel of Anti-C Sera Free From "Incomplete" Anti-D and with Anti-C^w, Anti-C^x, and Anti-c sera

	All with Papain														Saline		With Papain		
	Pure Anti-C							Anti-C ^w				Anti-C ^x			Anti-c		Anti-c		
	Bra	Bio	Cas	Fra	Gor	Sh800	Su740	Ams	Sid	BGRL	Kno	Tro	Mer		Har	Sh692	Sh720	New	Wig
R.D. Cc	+++	+++	+	+++	+++	+++	+	-	-	-	-	-	-	Cc	+++	+	w	+	+
Wat. cc	-	-	-	-	-	-	-	-	-	-	-	-	-	CC	-	-	-	-	-
E.B. C ^w c	+++	+++	-	w	-	+++	-	-	-	-	-	-	-	Miss Ch. C ^x C	-	-	-	-	-
Gsm. C ^w c	(+)	+++	++	-	+++	+++	-	+++	+	+++	+++	-	-	Mrs. W. C ^x C	-	-	-	-	-
Miss Ch. C ^x C	+++	+++	+++	+++	+++	+++	++	-	-	-	-	+++	+++						
Mr. Ch. C ^x c	w	+++	w	-	+++	+++	-	-	-	-	-	+++	+++						
*Miss H. C ^x c	-	+++	w	-	++	+++	(+)	-	-	-	-	+++	+++						
Miss E. C ^x c	w	-	w	-	w	+++	-	-	-	-	-	+++	+++						
Mrs. W. C ^x C	-	-	-	-	-	-	-	-	-	-	-	+++	+++						

* Frozen cell sample only.

An outstanding problem in the present case is the source of any immunization that may have occurred. The original antibody of Stratton and Renton resulted, like most known antibodies to antigens of the Rh series, from a straightforward immunization by a foetus which was subsequently born with haemolytic disease. In the present case the husband and surviving child lack the antigen; careful questioning has revealed no history of transfusion or injection of blood before the discovery of the antibody, or of any pregnancy by anyone but the husband. A spontaneous antibody would probably, however, unlike the present one, react better at 18° C. than at 37° C. and would not be greatly enhanced by albumin; a strong indirect anti-human-globulin test would, on the other hand, have been anticipated for an immune antibody.

One further piece of evidence that may have a bearing on the origin of the antibody is given by the serum Ga, which was discovered some years ago by Dr. M. M. Pickles and was at first thought to contain only anti-S. A recent investigation by Dr. R. R. Race shows that this serum also contains anti-C^x and anti-Mi^a. It is no longer possible to investigate the antigenic stimuli which may have given rise to these antibodies, but it may be that these two antibodies to rare antigens are, like anti-Wr^a, apt to arise without specific antigenic stimuli. We are indebted to Dr. Race for allowing us to mention these unpublished facts, which incidentally make the serum described in this paper the third example, not the second, of anti-C^x to be discovered.

The serum of Mrs. T. at first appeared to contain no abnormal antibody other than anti-C^x, but after the present paper had been written it was found to contain also an antibody against a rare antigen, possibly new, at present under investigation by Dr. M. Lauer, of Hamburg, and the staff of the Blood Group Reference Laboratory. A second serum containing the same antibody was found not to react with the cells of Mr. T. nor with those of three C^x-positives, including Mrs. W. In view of this finding it should be stated that all diagnoses of the C^x antigen mentioned in this paper were confirmed with the original anti-C^x serum of Stratton and Renton, which was shown not to contain the extra antibody.

Summary

A third example of the antibody anti-C^x is described, detected in the serum of an untransfused female patient in the course of compatibility tests performed prior to transfusion during surgical treatment for adenocarcinoma of the caecum. Details are given of serological tests on the red cells of the donor of the blood shown to be incompatible, and her family, together with those of comparative tests carried out with cells having a range of variants at the C locus (C, C^w, C^x, and c) and the corresponding antisera.

No source of immunization by pregnancy or injection of blood was established, and the antibody, despite some immune characteristics, was thought to be naturally occurring.

Our thanks are due to Dr. F. Kellett* and Mr. J. Clarke for their kind assistance and advice, and to Mr. D. K. Mulvany, the surgeon in charge of the case, for permission to investigate his patient.

REFERENCE

Stratton, F., and Renton, P. H. (1954). *Brit. med. J.*, 1, 962.

*Since this paper was written we have learned with deep regret of Dr. Kellett's sudden death on February 27, 1958.

Diabetes is the subject of the fifth *Conquest Pamphlet* issued by the Research Defence Society. The pamphlet answers in simple language common questions about the disease. Copies are obtainable from the society, 11, Chandos Street, Cavendish Square, London, W.1, price 6d. each.

MULTIPLE HETEROTOPIC SQUAMOUS-CELL CARCINOMAS

A CASE REPORT

BY

J. H. EVANS, M.D., M.R.C.P.

Medical Registrar, Royal Free Hospital, London

AND

A. POMERANCE, M.B., B.S.

Registrar, Department of Pathology, Royal Free Hospital, London

The purpose of this report is to record the occurrence in one patient of three heterotopic primary squamous-cell carcinomas and to discuss the case history in relation to modern views on multiple primary tumour formation.

Case Report

The patient, a housewife, was aged 71 years at the time of her death in April, 1957. Her father had died at the age of 70 of "cancer of the lung," but there was no other history of cancer in the family. The patient had never smoked more than two or three cigarettes a day, her alcohol consumption was negligible, and she had not lived abroad. Bilateral inguinal herniae were repaired when she was 50 years old. Since about this age she had had a winter cough productive of mucoid sputum, and this had always cleared up during the summer. She had had no other illnesses, and had received no radiotherapy until the appearance of her first carcinoma.

In August, 1948, she developed a nodule on the bridge of her nose. Shortly afterwards it ulcerated and she also noticed enlarged glands in the left side of her neck. She attended hospital four weeks after the appearance of the lesion. On examination she had koilonychia but no evidence of anaemia. On the skin of the bridge of the nose there was a circular ulcer a little less than 1 cm. in diameter, with raised firm edges; it was mobile over the deep structures, and lay just to the left of the midline. Two firm discrete lymph nodes in the upper left cervical group and the left pre-auricular lymph node were palpable. There was no other abnormal finding, and, in particular, the vocal cords were normal. Examination of the blood and urine showed no abnormality. Biopsy of the edge of the ulcer showed a moderately well differentiated squamous-cell carcinoma.

The lesion healed rapidly after superficial radiotherapy to a surface dose of 6,000 r at 90 kV applied over two weeks. The enlarged lymph nodes were removed by block dissection. No metastatic deposits were seen on microscopy of the excised nodes. The neck was therefore not irradiated.

In April, 1953, the patient complained of hoarseness of the voice of five months' duration. On indirect laryngoscopy the anterior part of the edge of the left vocal cord was slightly thickened. The epithelium was intact, the cords were mobile, but there were no enlarged cervical lymph nodes. The rest of the upper respiratory tract and the other systems were normal. The skin of the nose had healed. The blood Wassermann and Kahn reactions were negative. A radiograph of the paranasal air sinuses was normal. A chest radiograph showed bilateral apical pleural thickening and calcified foci in the right cardiophrenic, right hilar, and right paratracheal regions, but there was no evidence of recent or active pulmonary disease.

Over the next three months the lesion on the vocal cord was observed at frequent intervals, and it developed into a reddish sessile swelling on the anterior third of the left vocal cord. It did not ulcerate, and the cords remained mobile. Microscopy of tissue obtained by biopsy of this