of diseases and a tendency for urban patterns to be similar over very wide areas. Many urban epidemics tend to involve the rural population secondarily. Finally, there may be industrialization, which evokes its own elements in the disease-patterns. These industrial diseases are, like the rural diseases, often very closely related to the geographical background: an example is the pneumoconioses, which are related to the geography.

Conclusion

In developing our approach, the attention to assemblages rather than to individual parasites or diseases is very important—it would be described by the biologists as a synecological approach, concerned with the functional setting of organisms in relation to the other organisms, as opposed to an autecological approach, concerned with a single organism and its relations with the environment. Several workers have drawn attention to the pathology of social groups in their entirety. Ryle (1948) approached social pathology with the experience of a clinician: he rightly looked upon society as an organism which may be approached as such by the clinician as well as by the pathologist, the epidemiologist, and the psychiatrist.

Those who are sympathetic towards the philosophy of holism expounded by General Smuts (1936) will at once see that the synecological approach is also holistic. The lesson is that as soon as components have been sufficiently studied in isolation, they should then be set provisionally into their organized system or pattern and that the system should be studied as an organism in its entirety. Taking the analogous parasite-patterns of rodents, we have been enabled, from anomalies appearing in the patterns, to make inferences and plan investigations which would not otherwise have occurred to us. These anomalies could be detected only by studying the patterns as patterns and not by studying isolated parasites.

Summary

The whole assemblage of diseases from which a community suffers forms a pattern which can profitably be viewed as a whole. Those diseases caused by organisms form part of a general parasite-pattern. With examples of such patterns drawn from studies of rodents in Malaya, the parasite-patterns are considered to be decided largely by environment—that is, by the geographical background (which may be taken on a very small scale).

The distribution of diseases in different communities in different places is approached by a holistic study of the relationship borne by the entire disease-patterns of the communities to the social patterns and the geographical background. As a simple example, it is suggested that the disease-pattern in Somaliland is more or less evoked by the geography of the country. Further illustrations are drawn from malaria in Ceylon and from the typhus fevers.

In the earliest stages of man's occupation the link between geography and the disease-pattern appears to be very close. Man soon alters his environment, but the disease-patterns, while changing also, are still related broadly to the environment, and the change is largely one of emphasis. Urbanization, however, evokes urban patterns of disease, which tend to be widely distributed and uniform in nature, largely by interchange between urban environments, which are essentially the same everywhere. Industrialization evokes new elements, which again may become closely linked to the geographical background.

The methods and aims of medical geography, or medical ecology, are of interest even to those concerned with

preventive medicine in the most urbanized or stereotyped environments. A particular biological approach is here added to those which are already familiar.

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HAEMOLYTIC DISEASE OF THE NEWBORN CAUSED BY A NEW Rh ANTIBODY, ANTI-C^x

BY

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Haemolytic disease of the newborn commonly occurs in Rh-negative women who are delivered of Rh-positive children, where the mother has Rh antibodies in her serum. Cases are also seen in which Rh-positive mothers are delivered of children affected with this disease; in the majority of such cases the maternal serum contains Rh antibodies anti-E or anti-c. Antibodies to other blood-group antigens—for example, anti-Kell—may also be present in the maternal serum and give rise to this condition. Occasionally, haemolytic disease of the newborn is caused by antibodies to much rarer blood-group antigens; these may belong to the Rh series—for example, C^w—but a number of cases have been described in which the cause has been a family antigen.

The family antigens appear to be of two kinds—those which have been observed because they have given rise to haemolytic disease of the newborn, and those which have been found within a family but are not associated with the occurrence of this disease. Those which have caused haemolytic disease of the newborn have been described by Levine *et al.* (1951) (Miltenberger); by Holman (1953) (Wright); and by Davidsohn *et al.* (1953) (Berrens). These blood groups are confined to the members of the family in which the case of haemolytic disease of the newborn occurred.

The present case occurred in a third pregnancy, and was originally thought to be due to a family antigen, but subsequent investigations suggested that it was, in fact, a rare Rh agglutinin at the C-c locus, C^x. It is proposed in this paper to describe the clinical history of the case and certain serological findings, and to discuss their importance. Detailed serological work and family studies will be described in full elsewhere.

Clinical History

The mother had had two previous pregnancies: in 1947 a full-term normal male child, who is alive and well; and in 1950 a 7-months pregnancy resulting in a stillbirth.

In 1953 a full-term male child, weighing 8 lb. 2 oz. (3.7 kg.), was delivered normally. The child was jaundiced at birth, but this had faded by the third day. He was artificially fed and gained weight normally. He vomited twice during the first week of life, and on the tenth day was noticeably

paler. When 15 days old he vomited and was noted to be very pale and slightly icteric, and showed reluctance to feed. There was no pyrexia or evidence of infection; stools and urine were normal; there was no external bleeding or any signs of internal bleeding. On the 18th day he was admitted to St. Mary's Hospital under the care of Dr. G. Komrower, when he was found on examination to be pale, slightly icteric, and lethargic.

The umbilicus was healthy, and examination of the abdomen revealed enlargement of the liver and spleen three fingerbreadths below the costal margin. No abnormality was detected in the heart and lungs; eyes, head, ears, mouth, and palate were normal. No abnormality was detected in the central nervous system. There were no congenital abnormalities. The blood count was: red cells, 1,200,000 per c.mm.; Hb, 22% (3.3 g.); C.I., 0.91; white cells, 9,000 per c.mm.; normoblasts, 80 per 100 white cells, some showing mitosis. The direct Coombs test on the child's cells was positive. The Wassermann reaction carried out on the mother's blood was negative, and antenatally her blood had been typed as Rh-positive.

A transfusion of 60 ml. of compatible whole citrated blood was given when the child was admitted, and the haemoglobin rose to 43%. On the 19th day after birth 90 ml. of group A Rh-positive compatible blood was transfused, and the haemoglobin increased to 59% (8.7 g.). On the 20th day the direct Coombs test was negative, and a further 100 ml. of blood was given; on the next day the haemoglobin was 72%, and the child was much improved. By the 26th day the haemoglobin had dropped to 63% (9.3 g.); 120 ml. of blood was given on the 27th day, and again five days later. The haemoglobin was now 93% (13.6 g.), and after this the child progressed well, and when 6 weeks old was in good health, both liver and spleen being impalpable.

Materials and Methods

Blood samples were obtained occasionally as citrated blood, but largely in the form of clotted blood; all random blood samples were obtained as clotted blood.

Methods.—Saline and albumin agglutination tests and titrations and the antiglobulin (Coombs) test were performed according to the techniques suggested by the Medical Research Council (1952). Papainization of the red cells was performed by the method described by Stratton (1953) slightly modified in order to increase the degree of papainization of the erythrocytes.

Serological Findings

Family Blood Groups

Below are shown the blood groups of the mother, the affected child, the father, and the healthy child of the family in which this case of haemolytic disease occurred:

Mother: A₁ R₁R₂ (CDe/cDe) M.S. P+ Le(a-b+) Lu(a+),K- Fy(a+) Cx-
 Affected child: A₁ R₁R₂ (CDe/cDe) M.N.S. P- Le(a-b-) Lu(a+),K- Fy(a-) Cx+
 Father: A₁ R₁r (CDe/cde) M.N.S. P+ Le(a-b+) Lu(a+),K- Fy(a+) Cx+
 1st child: A₁ R₁r (cDE/cde) M.N.S. P+ Le(a-b-) Lu(a+),K- Fy(a+) Cx-

All members of the family were tested with the maternal serum. The father and the affected child were positive, but the first child and, of course, the mother were negative. The maternal serum was titrated by various techniques with the father's cells. The results are shown in Table I.

TABLE I.—Results of Titrating the Maternal Serum Against the Father's Cells

Cells	Method				
	Saline Agglutination		Albumin Agglutination	Coombs Test	Papainized Cells
	20° C.	37° C.	37° C.	37° C.	37° C.
Father ..	Neg.	1:1	1:4	1:8	1:64

Investigations on Maternal Serum

The maternal serum was tested with a series of Group O cells known to contain the antigens C,D,E, c,d,e, C^w, C^u, M,N,S,s,P, Le^a, Le^b, Lu, K, Fy^a, Fy^b, Jk^a, Jk^b, Levay positive cells (Callender and Race, 1946), and Wright positive cells (Holman, 1953). Negative results were obtained, using all techniques. The serum was also similarly tested with:

11 samples of blood of genotype rr	(cde/cde)
23 " " " " "	R ₁ r (CDe/cde)
6 " " " " "	R ₁ R ₂ (CDe/cDe)
7 " " " " "	R ₁ r (cDE/cde)
2 " " " " "	R ₁ R ₂ (cDE/cDe)
3 " " " " "	R ₁ r (cDE/cde)

and with blood samples of genotype R'r (Cde/cde), R'r (cDE/cde), R^w₁r (C^wDe/cde), R₁r (CdE/cde), R^u₁r (CD^ue/cde), R^u₂r (cD^uE/cde), and -D-/-D-.

The maternal serum was further tested with a total of 3,931 blood samples from healthy blood donors. These were selected only by reason of their ABO groups, and they comprised 1,727 Group A, 1,915 Group O, 210 Group B, and 79 Group AB.

Initial testing of 267 samples was performed, using the tube albumin technique at 37° C., but the remainder of the tests were made with the papainized-cell technique in capillaries at 37° C. (Stratton, 1953). Control tests were used, each time employing a known positive reactor, and strong agglutination was obtained on each occasion. The Group O and A samples were tested with unabsorbed serum; the Group B and AB samples were tested with serum from which the anti-B agglutinins had been absorbed with papainized Group B Rh-positive cells. Four positive results were obtained when the 3,931 samples of blood were tested with the maternal serum; these were the 820th, 995th, 1,286th, and 1,779th samples tested. In each case the cell gave positive results with the maternal serum, using the albumin and Coombs techniques, as well as by the papainized-cell method. The four cell samples were tested further, with the following results:

Sample 1 (Lo): A₂ R₁r M.S. P+ Le(a-b-) Lu(a-) K- Fy(a+) Cx+
 " 2 (Os): O R₁r M.S. P+ Le(a-b+) Lu(a-) K- Fy(a-) Cx+
 " 3 (Ho): O R₁R₂ M.N.S. P+ Le(a-b+) Lu(a-) K- Fy(a-) Cx+
 " 4 (Pri): A₁ R₁r M.S. P- Le(a-b-) Lu(a-) K- Fy(a-) Cx+

Each of these cell samples was shown to have a negative direct Coombs reaction. Tests on these samples and the father's cells with 20 fresh normal sera gave negative agglutination reactions in saline at 20° C. and 37° C.; polyagglutinability was thus excluded. The presence of the antigen A₄ was excluded in the case of the Group O proposition by testing them with the sera from Group O persons known to give strong reactions with A₄ cells. The father's cells and one of the proposition also gave a negative result when tested with anti-C^w, anti-Wright (Holman, 1953), anti-Miltnerberger (Levine *et al.*, 1951), anti-Black, anti-Berrens (Davidsohn *et al.*, 1953), and anti-Graydon (Graydon, 1946) sera.

Investigation of Cell Samples Giving a Positive Result With the Maternal Serum

The father's cells and the four other cell samples described above were titrated with five anti-C typing sera in saline at 37° C. In Table II are shown the titres which were obtained by testing the sera with these cells, and with a number of cells containing the C agglutinin, the latter giving negative results with the maternal serum of this case. It will be observed that several of the sera give much lower titres than they do with the control cells of genotype R₁r (CDe/cde) or R₁R₂ (CDe/cDe), although with one anti-C serum the results of testing the control cells and those giving a positive result with the maternal serum do not differ from one another. The detailed scoring of the titration of one of these anti-C sera with cells which gave positive results with the maternal serum, and with two control cells which did not, are shown in Table III. It will be seen that not only in certain cases are the titres lower, but the individual reactions between the various dilutions of the serum and the cells are weaker. Hence the titrations

TABLE II.—*Titration of Anti-C + Incomplete Anti-D Sera, Using Various Cells*

Cells	Rh Type	Result with Maternal Serum	Sera				
			Anti-C (i)	Anti-C (ii)	Anti-C (iii)	Anti-C (iv)	Anti-C (v)
Father	R ₁ r (CDe/cde)	+	1	1	16	2	4
Lo	R ₁ r (CDe/cde)	+	2	2	8	1	1
Pri	R ₁ r (CDe/cde)	+	2	2	16	4	2
Os	R ₁ r (CDe/cde)	+	4	2	16	4	4
Ho	R ₁ R ₂ (CDe/cDe)	+	4	2	16	2	2
A	R ₁ r (CDe/cde)	—	64	8	8	32	16
B	R ₁ R ₂ (CDe/cDe)	—	32	2	16	16	8
C	R ₁ R ₁ (CDe/cDe)	—	32	8	16	32	16
D	rr (cde/cde)	—	—	—	—	—	—
E	R ₂ R ₂ (cDe/cDe)	—	—	—	—	—	—

TABLE III.—*Results of Titration of Anti-C (i) with Normal Cells and Cells Positive with Maternal Serum*

Cells	Results with Maternal Serum								Control
		1:1	1:2	1:4	1:8	1:16	1:32	1:64	
R ₁ r (CDe/cde)	—	+	+	+	+	+	+	+	—
R ₁ R ₁ (CDe/cDe)	—	+	+	+	+	+	+	+	—
Father	+	+	+	+	+	+	+	+	—
Lo	+	+	+	+	+	+	+	+	—
Pri	+	+	+	+	+	+	+	+	—
Os	+	+	+	+	+	+	+	+	—
Ho	+	+	+	+	+	+	+	+	—

with the control cells and with cells containing this special C antigen, as shown in Table III, have a characteristic pattern of reaction. Titration of anti-c showed that the father's cells contained only a single dose of c-agglutinin.

Family Studies

The original family in which the case occurred has been studied as well as four other families. In these families it has been shown that the cells of members of the families which gave positive results with the maternal serum of the above case were all of type R₁r (CDe/cde), R₁R₂ (CDe/cDe), or R₁R₁ (CDe/cDe). Where the members of the families were of type R₁r or R₁R₂ the C antigen showed a similar modification to that described above, but in cases of type R₁R₁ the modification was masked by the presence of a normal C in the red cell. The family studies were consistent with this new Rh antigen (C^x) being inherited as a Mendelian dominant character.

Discussion

When this case first came to our notice it might not have received detailed consideration if it had not been for two factors. The first of these was the fact that the child was a severely affected case of haemolytic disease of the newborn and the diagnosis was not in doubt. The child's cells gave a strongly positive direct Coombs reaction when first examined on the 18th day. The second factor was that it is customary in this laboratory to make direct tests between the husband's cells and the maternal serum in those cases in which there is a suspicious history and in which no antibodies are found in the maternal serum by the usual methods of testing with standard cells. These include tests by saline and albumin agglutination techniques, by the Coombs technique, and using papainized red cells.

Following the direct tests between the husband's cells and the maternal serum the antibody was detected. It was obvious that this was an antibody of very unusual character, and consideration was given to the possibility of its reacting with a family antigen similar to others that had been described. This was thought to be especially likely when it was found that negative results were obtained with the usual test cells that were known to contain all blood-group antigens. The maternal antibody, nevertheless, was

found to give strong positive results with the father's cells when these were treated with papain; the titre in this latter case was 1:64, whereas using the anti-globulin technique it was only 1:8. Several blood-group antibodies are known to give strong positive results with papainized red cells positive for the homologous antigen when they are prepared by the method of Stratton (1953). Since this was an ABO compatible pregnancy, and since Lewis and P antibodies are not known to be associated with haemolytic disease of the newborn, our attention was directed to the possibility of some antibody of the Rh series, or possibly a variety of anti-S or, on the other hand, some unknown antibody, which was responsible for this strong positive result when papainized cells were employed.

Tests for the rare Rh antibody, anti-C^w, proved to be negative, and when the maternal serum was tested with cells of type C^uDe/cde—a very uncommon type of cell (kindly provided by Dr. R. R. Race)—the results were also negative.

Our attention was further directed to the Rh antigen by the fact that when the father's cells were first typed they were routinely typed as R₀r (cDe/cde), and it was only on further examination, using several anti-C typing sera, that they were found to be of type R₁r (CDe/cde). It is not proposed to go into details in this paper concerning the reactions between these cells and various anti-C sera except to say that the cells appeared to have a modified C antigen which gave characteristic reactions. Cells which gave positive results with the maternal serum all gave this characteristic pattern of reaction except where this was masked by the presence of a normal C antigen, as in the genotype CDe/C^xDe. Had it not been for this characteristic pattern of reaction with various anti-C typing sera and the strong reaction which the maternal serum gave with papainized red cells we might not have thought the antibody to be one associated with the Rh blood group, but might have assumed it was reacting with a family antigen. This latter contingency was quite likely, since when testing random cell samples the first positive result was not obtained until 820 blood samples had been tested. These results suggested, therefore, that we were identifying an allele of Rh at the C-c locus. Moreover, family studies suggested that this was inherited as a Mendelian dominant character. That the serum of the mother was recognizing an allelic form of C could no longer be doubted when it was found with the peculiar form of C and the antigen recognized by the maternal serum showed absolute linkage in the family studies.

This antigen, which we proposed to call "C^x," appears to be analogous to C^w and to be a modification or mutation of C at the C-c locus. Unlike C^u, D^u, and E^u, but like C^w, it has an antibody which reacts with it specifically and characteristically. Anti-C^w antibodies occur in persons of genotype R₁r (CDe/cde), and similarly in this case a mother of genotype R₁R₂ (CDe/cDe) was immunized by a father of genotype C^xDe/cde. It is interesting to note that the difference between C^x and C is sufficient to cause immunization of the mother and the child to be affected with haemolytic disease of the newborn. Although this is the first C^x-positive living child that the mother has borne it is not necessarily the first antigenic stimulus, however, since the second pregnancy resulted in a stillbirth.

This case brings to our attention certain important factors. The first is that when a suspicious case of haemolytic disease of the newborn is observed, or where the clinical history suggests this diagnosis—for example, when there is a history of children affected with jaundice or anaemia or hydrops, or of multiple stillbirths—if no atypical maternal antibodies are found by routine methods then a direct test should be made between the father's cells and the mother's serum. The other interesting feature of this case is that the immunization of the mother might so readily have been thought to be due to a family antigen if the testing of a large number of random cell samples had not been undertaken. From our experience in this case it would seem that very large numbers of random tests need to be made before

one can conclude that an antigen is a true family antigen. Particularly is this so if there is the possibility of immunization to an antigen of the Rh series.

This antigen is very infrequent in the English population; it might be that in other populations the frequency would be greater.

Summary

A description is given of a case of haemolytic disease of the newborn due to a new Rh antibody, anti-C^x.

Anti-C^x antibody gives very few positive results when tested with random blood samples from the English population. Out of 3,931 random samples tested, four positive results were obtained.

The C^x antigen is considered to be an allele of Rh at the C-c locus. Its inheritance has been demonstrated.

The desirability of including direct tests between the husband's cells and the maternal serum in suspected cases of haemolytic disease, as well as the need for extensive investigations on antibodies thought to be reacting with family blood groups, is discussed.

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PORPHYRIN EXCRETION IN A PATIENT WITH DELAYED LIGHT- SENSITIVE PORPHYRIA

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Extensive knowledge based on modern techniques is becoming available on the porphyrin excretion in patients with acute porphyria (Nicholas and Rimington, 1953), but the present state of knowledge on the porphyrins of "delayed light-sensitive porphyria" is much less advanced. This term has been used (Prunty, 1952) in a suggested modified classification of porphyria to include cases referred to by previous authors as "porphyria cutanea tarda." The condition usually occurs in men of adult age and is not familial. Amongst the other changes, one important clinical manifestation is a bullous eruption of the skin produced by light and trauma. The present paper concerns additional data obtained from one of the rare cases of this condition seen by chance in this country.

Clinical Description of the Patient

A man aged 54, normally resident in South Africa, was admitted to St. Thomas's Hospital for investigation. He complained that he had had blisters on his hands for the past year. He has also suffered from arthralgia of the left hip, left knee, right ankle, and right shoulder for three years. There was no relevant family history. He had had blackwater fever in 1915, appendicectomy in 1924, and a cholecystectomy in 1945 for an illness accompanied by abdominal pain and jaundice. In 1952 radiological evidence of the presence of colonic diverticula had been obtained; at about this time he had had symptoms of diverticulitis. Apparently the porphyria became manifest a year before admission. Recurrent crops of blisters about 0.5 cm. in diameter occurred on the backs of his hands and fingers. They took four or five days to develop and then burst. They often contained a clear reddish fluid. This blistering did not appear to be related to exposure to sunlight. He had been treated with a sulphonamide for the diverticulitis without influencing the skin condition. There was some evidence that trauma to the skin favoured the production of these blisters. During the year his urine had been the colour of "dark ale," and had occasionally been dark during the last four years.

Physical examination showed a number of small circular scars on the backs of the hands and fingers, with a circular zone of slight pigmentation around some of them. When first seen three weeks before admission he had three blisters about 0.25 cm. in diameter on his hands, containing yellowish fluid, but these had disappeared by the time of his admission. There was a small rodent ulcer on the right cheek. The liver was enlarged one fingerbreadth below the costal margin, and scars from his two abdominal operations were present. There was a minor degree of osteoarthritis in the knees, hips, and right shoulder.

His urine on examination had a rosy hue and not the brownish-purple appearance of acute porphyria urine containing porphobilin. Porphobilinogen was never detected. On three consecutive days his pigment excretion measured:

Day	Urobilinogen mg./day	Total Porphyrin mg./day	Total Porphyrin Minus Uroporphyrin µg./day
1	9.4	2.4	37
2	6.6	4.8	80
3	3.0	4.4	126

The serum and cerebrospinal fluid contained no porphyrin. Faecal urobilinogen excretion was 295 mg. a day—a normal quantity. Serum total protein was 7.7 g. per 100 ml. (albumin 4.3 g. and globulin 3.4 g.). Thymol turbidity was 4 units; serum bilirubin 0.7 mg. per 100 ml.; cholesterol 159 mg. per 100 ml., of which 65% was esterified. The prothrombin time was 12 seconds (normal). The blood urea was 21 mg. per 100 ml., and endogenous creatine clearance 83 ml. a minute. The haemoglobin was 16.2 g. per 100 ml., and the packed cell volume 48%.

Urine Porphyrin

The porphyrin was isolated as previously described (McSwiney, Nicholas, and Prunty, 1950). Chromatography of the esters on columns of Al₂O₃ (Savory and Moore) and MgO Grade III (Nicholas, 1951) (see Diagram) to clean up the porphyrin gave a single main band (1), which on further chromatography on MgO Grade IV yielded a main band (1a) and traces of two other bands. This main fraction was crystallized, and the product had a melting-point at 211 to 213° C., and its α band at 624.3 m μ . Each band obtained from these three columns was submitted to paper chromatographic analysis, with the results shown in the Diagram. It should be noted that most of the urinary porphyrin was lost during preliminary purification.

There were two very faint bands, 1c and 1e, too little for any paper chromatography at all.