A "NEW" Rh ANTIBODY, ANTI-f

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A "new" Rh antibody, which we propose to call anti-f, has been found in the serum of a man, Mr. Cl., aged 30, who suffers from haemophilia.

Since childhood Mr. Cl. has received about 35 transfusions of blood, the last of which, in December, 1951, was followed by a shaking chill. He was seen by us, for the first time, in March, 1952,* when three blood-group antibodies were found in his serum—anti-B, anti-K, and anti-S. In addition there was at least one other unidentified antibody. In the hope of clarifying the reactions of this antibody 0.25 ml. of O, NsNs, cDe/cde, kk blood was injected on two occasions; following this, the presence of anti-N was disclosed and, in addition, the antibody anti-f, which is the main reason for this publication.

The blood groups of Mr. Cl. are: A₁, MsMs, P+, CDe/cDE, Lu(a-), K-, Le(a+b-), Fy(a+), Jk(a+b+).

The serum of Mr. Cl. can be absorbed free of the other antibodies, leaving anti-f alone. This absorbed serum has been used to test the blood of 276 unrelated white people and 114 of their relatives. From the results it is clear that the antigen recognized by anti-f is part of the Rh complex of antigens; it is also clear that the antigen is not associated with any other of the known blood-group antigens, with sex, or with the ability to taste phenylthiocarbamide.

Properties

In brief, the antibody reacts with a product of the chromosomes cde and cDe, which may consequently be distinguished by the addition of f. We have been able to classify all the important chromosomes save the rare cD^ue (which we expect to have f).

If the absence of f is indicated by the letter F the chromosomes are:

cdef, cDef.

CDeF, cDEF, C^wDeF, CdeF, cDEF, CD^ueF, cD^uEF, CDEF, CD^uEF, C^wdeF, and CdeF.

These are the usual Caucasian chromosomes. For reasons which will be given in a later communication, we expect certain less common combinations to exist—namely, cDeF, cdeF, CDeF, and cDEF. We have found a sample of blood giving the reactions expected of cDeF/cdeF and one giving the reactions expected of cdeF/cdeF. If the genes are arranged in a linear order on the chromosome then the order DCEF fits best with the observed facts.

If anti-F exists, as we presume it does, it will be difficult to identify with the test cells commonly available: it will give the reactions of a mixture of anti-C and anti-E, and, furthermore, it may be combined also with anti-D.

Anti-f agglutinates red cells suspended in albumin, and the results can be confirmed by absorption tests. The reaction of homozygous, ff, cells is stronger than that of heterozygous, Ff, cells. The antiglobulin test is not particularly useful with this example of this antibody.

Anti-f does not promise to be of any great practical importance in routine Rh blood-group work. Certainly it splits off CDe/cde and CDe/cDe from CDe/cDE, CDe/cdE, and Cde/cDE; it also separates CDe/cde from Cde/cdE. But these are hardly day-to-day problems. There is evidence that anti-f may be present in some anti-e and anti-c sera, and so these sera are laid open to investigation from this angle.

From a theoretical point of view anti-f is obviously of the first importance and must be taken into account in any thought about the Rh groups. Many interpretations of this new antibody have been considered, but the reactions can be most simply and conservatively explained by the postulation of a fourth allelomorphic pair of Rh antigens, f and F.

A detailed account of the serological and genetical work on anti-f will be published soon, together with acknowledgment to many people who have helped us by collecting samples of blood of rare Rh genotypes.

THREE CASES OF SUPPURATION DUE TO FUSIFORMIS INFECTION

BY

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A number of non-sporing, non-motile, anaerobic bacilli have been isolated by different workers from necrotic lesions in man and animals. They represent an ill-defined group of organisms classified at the moment as the genus *Fusiformis* (Topley, Wilson, and Miles, 1948). The term "necrobacillosis" has been given to the disease they cause in man.

These organisms have been demonstrated in the normal flora of the mouth, intestines, and genito-urinary tract by Beveridge (1934) and others, and in a variety of human pathological lesions, including ulcerative colitis (Dack, Dragstedt, and Heinz, 1936), in osteomyelitis and mastoiditis (Chandler and Breaks, 1941), in infected wounds (Forbes and Goligher, 1944), in post-abortive fever (Jones, 1944), in puerperal fever (Harris and Brown, 1927), and in appendicitis (Veillon and Zuber, quoted by Topley *et al.*, 1948). These organisms have also been isolated from various necrotic conditions in animals, such as calf diphtheria and labial necrosis of rabbits. A fuller description of these organisms is given by Beveridge (1934), Lemierre (1936), and Schwabacher and Mitchison (1947).

Organisms of the *Fusiformis* group were isolated from the following cases of peritonitis (two cases) and ischio-rectal abscess (one case), and the characteristics of the organisms were substantially the same in all.

Case Reports

Case 1.—A married woman aged 45 was admitted to hospital on March 14, 1950, complaining of pain and tenderness in the right groin for the past eight days. On examination the abdomen was distended and shifting dullness was present. A large ill-defined mass was felt in the right loin and the overlying skin was red and oedematous. Laparotomy was performed next day and general peritonitis was

*Referred by the Hemophilia Foundation, Inc.

found with many adhesions. Gentle exploration of the upper right abdominal quadrant liberated a gush of foul-smelling pus, and 2 pints (1.1 litres) was removed by suction, a drain was inserted, and the abdomen was closed. A provisional diagnosis of appendix abscess was made at this time. A course of penicillin, 250,000 units twice a day, and streptomycin, 0.5 g. once a day, was started. In spite of this her temperature did not settle and a further operation was performed on March 23. At this a further 2 pints (1.1 litres) of pus was evacuated through a right subcostal incision, presumably coming from a subphrenic abscess. The patient slowly improved after this, although foul-smelling pus continued to be discharged from her wound for several days. The streptomycin was discontinued after nine days and the penicillin after two weeks, when a total of 6,250,000 units had been given. The patient made a slow but satisfactory recovery, and was discharged a few weeks later.

Case 2.—A man aged 64 came to the out-patients' department complaining of a painful lump on his buttock. On examination an ischio-rectal abscess was discovered. This was incised and about 4 oz. (114 ml.) of foul-smelling pus was evacuated. A drain was inserted, an injection of 1 ml. of "prolophen" was given, and the patient was sent home to rest. A further injection of prolophen was given next day, and the dressing was changed. Two days later he was feeling well and comfortable, and his abscess was discharging through the incision. A week later the wound was healed and the patient was completely cured.

Case 3.—A man aged 43 complained of sudden onset of pain in the lower abdomen. On examination he was pyrexial and there was generalized abdominal rigidity with tenderness in the right iliac fossa. A diagnosis of perforated abdominal viscus was made, and a laparotomy was performed the same day. Free pus was found in the peritoneal cavity, and the appendix was acutely inflamed and had perforated. The appendix was removed and a suitable drain inserted. Penicillin, 500,000 units a day, was given for seven days (a total of 3,900,000 units). Eleven days after admission he began to have rigors and a mass could be felt in the right loin. A second operation was performed, at which $\frac{1}{2}$ pint (285 ml.) of foul-smelling pus was released under pressure from the right paracolic gutter. Two drainage tubes were put in, and a course of chloramphenicol was begun, 8.5 g. being given in five days. The patient made an uneventful recovery.

Bacteriology

In all three cases foul-smelling dirty-brown pus was obtained from the abscesses. Films stained by Gram's method showed pus ++, necrotic debris, and some small Gram-negative bacilli. In addition, in Case 2 some Gram-positive cocci and in Case 3 both Gram-positive cocci and large Gram-negative bacilli were also seen. The material was inoculated on to blood-agar plates and incubated aerobically and anaerobically.

The aerobic plates in Case 1 were sterile, in Case 2 they grew a few colonies of *Staph. saprophyticus* and *Bact. coli*, and in Case 3 *Bact. coli* only. After 24 hours' incubation the anaerobic plates in all cases showed some very small pin-point colonies resembling those of *Haemophilus influenzae*. After 48 hours the colonies measured 0.5–1 mm. in diameter, were smooth and convex, and were slightly opaque like drops of milky water. Stained films showed them to be composed of slender pleomorphic Gram-negative cocco-bacilli, some the size of *H. influenzae* and others as large as coliforms. There were also a few slender filamentous forms. A small zone of haemolysis was now apparent surrounding the colonies. The organisms were subcultured on to blood agar, plain nutrient agar, and into Robertson's meat broth and incubated aerobically and anaerobically. All aerobic plates remained sterile, but the organism grew satisfactorily on blood agar and plain agar under anaerobic conditions, and it also grew in Robertson's broth, from which it was again subcultured on to blood agar and grown anaerobically. At no time was the organism grown under aerobic conditions. The addition of a few

drops of laked horse cells (to supply the X factor) or streaking the plate with *Staph. pyogenes* (to supply the Y factor) did not enable the organism to grow aerobically, but the growth on the anaerobic plates appeared to be improved by their addition, and "satellitism" around the staphylococcal colonies occurred.

In Case 3 an anaerobic streptococcus was grown as well as the fusiformis. The association of anaerobic streptococci with these organisms has already been quoted by Lemierre (1936). The organisms were kept going quite easily by subculturing every two or three days on to fresh blood-agar plates. Acid but no gas was produced in lactose, glucose, maltose, and saccharose, though not in dulcitol or mannite. Two or three days' incubation in an anaerobic jar was required to produce a satisfactory growth in the sugars. The addition of serum to the sugar media did not appear to be necessary. Indole was produced by Case 3 but not by the other two. Gelatin was not liquefied by any of the organisms, and the reaction in milk was neutral. The organism from Case 3 was found to be sensitive to penicillin, streptomycin, and chloramphenicol; the others were not tested.

Broth cultures of the organisms from Cases 1 and 2 were inoculated subcutaneously into guinea-pigs with no apparent ill effect. The animals were killed four weeks later and no abnormality was discovered at the post-mortem examination. Unfortunately no rabbits were available at this time.

Discussion

The organisms from these three cases had very similar cultural and biochemical characteristics, which, so far as they went, also agreed with the descriptions by Schwabacher and Mitchison (1947) for two strains of the genus *Fusiformis* and by Topley *et al.* (1948) for *Fusiformis fragilis*. The organism from Case 3 differed from the others by producing indole, but Chandler and Breaks (1941), Forbes and Goligher (1944), and Owen and Spink (1948) all described indole production in their cases.

There is no doubt that there must be many strains in this group, as various individuals have reported different characteristics, but it is thought that the organisms described in this paper can reasonably be included in the genus *Fusiformis*—the main characteristics of the group being that they are non-sporing, non-motile, anaerobic Gram-negative cocco-bacilli which show a variable amount of pleomorphism and filamentous forms. The material from which they are originally obtained is usually foul-smelling pus. Colonial and morphological appearances resemble the *Haemophilus* group, but their ability to grow without X and Y factors, and their dependence on strict anaerobic conditions, exclude them from this group.

In dealing with material from the female genital tract it is important not to confuse fusiformis organisms with morphologically similar Gram-negative cocco-bacilli, which can often be grown from the normal vagina. These latter organisms are not true anaerobes and will grow aerobically as long as X and Y factors are present, but will not grow on plain agar. Moreover, the colonies are smaller and do not show the "milky water" appearance of fusiformis. As they can be found in the flora of the normal vagina of healthy adults and young girls, they are not regarded as pathogenic, and their dependence on X and Y factors for growth suggests that they may be members of the *Haemophilus* group, possibly related to *H. ducreyi*.

The three cases of necrobacillosis described in this paper all recovered, and the one organism tested was found to be sensitive to penicillin, streptomycin, and chloramphenicol. It therefore seems that with modern antibiotics, and surgical drainage where indicated, the prognosis in these cases is good.

Summary

Three cases of suppuration due to infection by fusiformis organisms are described. All made satisfactory recoveries.

The morphological and cultural characteristics of the organism are described, and are compared with those reported by other workers.

A morphologically similar organism found in the normal vagina, which must not be mistaken for fusiformis, is briefly mentioned.

With suitable antibiotic treatment, and surgical drainage where indicated, the prognosis in infection with these organisms is regarded as good.

I wish to thank both Dr. J. L. Pinniger, of St. Thomas's Hospital, London, in whose laboratory this work was done, and Dr. Mary Barber, of the Department of Bacteriology, St. Thomas's Hospital Medical School, for their helpful criticism and advice.

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EXPERIMENT TO DETERMINE IF A PROGUANIL-RESISTANT STRAIN OF *P. FALCIPARUM* WOULD RESPOND TO LARGE DOSES OF PYRIMETHAMINE

BY

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This experiment was undertaken to find out if a proguanil-resistant strain of malaria also showed partial or absolute resistance to pyrimethamine ("daraprim"). I was both subject and experimenter. By the co-operation of Dr. G. I. Robertson, of the I.C.I. laboratory, Nairobi, I received 10 bites by five mosquitoes (*A. gambiae*) infected with a proguanil-resistant strain of malaria parasite imported from the Tampin area in Malaya.

The biting took place on August 21, 1952. All mosquitoes showed sporozoites on dissection. Parasites appeared in the blood on September 1 (Day No. 1) (see Table).

Course of the Infection

Day No.	Maximum Parasitaemia	Ring/Leucocyte Ratio	Treatment	Time of Treatment
1	38/50 leucocytes	0.7:1	50 mg. pyrimethamine	Noon
2	53/50 "	1:1	50 " "	"
3	94/50 "	2:1	100 " "	"
			100 " "	2 p.m.
			50 " "	10 "
4	625/50 "	12:1	10 gr. quinine i.v., 7 tab. chloroquine in divided doses	1 "
5	763/50 "	15:1	10 gr. quinine i.v. and 10 gr. by mouth, 6 tab. chloroquine in divided doses	1 "
6	58/50 "	1:1	4 tab. chloroquine and 20 gr. quinine in divided doses	

On the third and fourth days the white blood cell counts were 4,100 and 4,500 per c.mm. respectively. After the sixth day recovery was rapid, and I was able to leave hospital on the tenth day feeling fit. At no time was toxæmia profound, and mental clarity was retained throughout, though headache became very severe on the fourth day.

Discussion

I had not previously had malaria, so immunity played no part in the infection. The experiment proved that a strain of malaria resistant to proguanil can show absolute resistance to pyrimethamine. This is hardly surprising when it is realized that pyrimethamine resulted from research on the antimalarial properties of diaminopyrimidines undertaken because these compounds showed a structural analogy with proguanil and similarities of microbiological behaviour. It is probable that both drugs act upon the same enzyme system of the malaria parasite.

It is evident that medical policy in Kenya has been wise, in that the African population has not been subjected to treatment with proguanil on a large scale. Because of dispensing difficulties in Reserves this might have been given in doses small enough to allow the widespread rise of resistant strains. The same applies, in theory, to pyrimethamine, though the drug is effective in such small doses that this danger seems unlikely to occur. The sale of 5 mg. tablets for prophylaxis would be dangerous, as adult Africans would use them for treatment, with the certainty of underdosage. Perhaps only tablets of not less than 25 mg. should be allowed for sale. These are readily divisible for treating infants, and, as the drug has a very low toxicity, there seems little to fear from overdosage. Generally speaking, it is probable that the majority of people taking pyrimethamine will do so in doses that, though harmless, are far more than actually required to eradicate infection. The fact that the drug is tasteless and cheap should ensure this.

In a recent paper Wilson and Edeson (1953), as a result of work in Malaya, report no difference in response to pyrimethamine between unselected strains of malaria and those resistant to proguanil. East African strains of malaria parasites are extremely sensitive to pyrimethamine, though recently a case of subtertian malaria which did not respond to pyrimethamine has been encountered, the resistance of the infecting parasite being confirmed by subinoculation. It would be a tragedy if other resistant strains were imported. The presence of men of the King's African Rifles in Malaya creates a hazard, and it is hoped that special care will be taken that none return to East Africa with the gametocytes of Malayan strains of malaria in the blood.

I thank the Director of Medical Services, Kenya, for permission to publish this paper and Dr. B. P. Harris for undertaking treatment at the conclusion of the experiment, when intervention became necessary; thanks are also due to the senior parasitologist, Dr. R. B. Heisch, who watched the parasite rate when I was unable to do so, and to Dr. G. I. Robertson for the great help and interest shown by him in the experiment. The nursing skill and attention were beyond praise.

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This year the number of students applying for admission to American medical schools has again declined, according to a survey by the director of studies for the Association of American Medical Colleges. 3,150 fewer students applied this year than in 1951-2, 7,600 less than three years ago. Of the 16,763 persons applying last autumn only 7,778 were accepted. The survey also shows that the average pre-medical student applies to three or four medical schools, and that the geographical distribution of students is uneven, half the candidates coming from only seven States. One cautious student applied to 45 medical schools and was accepted by one.