normal rabbit kidneys were found to contain a strong inhibitor of human erythropoietin obtained from human urine or from human plasma. The kinetics of the inactivation suggests it to be a nonenzymatic process. Dialysis against water or EDTA saline did not change the activity of the inhibitor and heating to 100°C did not completely abolish its activity. The addition of normal plasma reduced the inhibitory activity of the renal homogenate, a reduction that was directly proportional to the amount of normal plasma added and was observed both with urinary erythropoietin and with plasma erythropoietin. Homogenates prepared from normal rabbit spleens and livers were also found to contain an inhibitory principle that was modified by the addition of normal plasma. Homogenates from normal rabbit bone marrow were almost devoid of inhibitory activity.

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Hemagglutinins in Sera of Africans of Gambia* (33440)

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Thirty-eight sera collected from Africans in the Medical Research Council Laboratory at Fajara, Gambia, British West Africa were studied for antierythrocyte activity. This report brings results obtained by testing these sera against (i) mammalian erythrocytes of foreign species origin, (ii) human blood group A and B erythrocytes, and (iii) human blood group O erythrocytes exposed to trypsin.

Methods. For titrations, twofold increasing saline dilutions of the sera were prepared in 0.1-ml volumes. To each test tube was added 0.1 ml of 1% suspension of three-times washed erythrocytes. Following 2 hr of incubation at room temperature, the test tubes were centrifuged at 500 rpm for 2 min and agglutination was recorded after shaking the test tubes gently. The titer was expressed as the reciprocal of the highest serum dilution at which definite agglutination was noted.

Trypsinized erythrocytes were prepared by mixing 10 vol of 2% erythrocyte suspension with 1 vol of 1% trypsin solution. The mixture was incubated at 37° for 10 min. Thereafter the erythrocytes were washed with cold saline.

For the indirect Coombs' test, the test serum, at various dilutions, was mixed with an equal volume of a 1% erythrocyte suspension. The mixture was incubated for 1 hr at room temperature. Thereafter the erythrocytes were washed 3 times with saline and resuspended to 1% concentration. One-tenth of this suspension was mixed with 0.1 ml of a Coombs' serum at a proper dilution, and

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TABLE I. Hemagglutination by Gambian (I) and Normal American (II) Sera.

		No. of sera with a titer of							
		≤20	40	80	160	320	640	≥1280	Total
Guinea pig hemagglutinins	I	12	4	5	10	3	3	1	38
	II	24	3	0	1	0	0	0	28
Rat hemagglutinins	I	9	4	5	8	4	5	3	38
	II	48	30	7	1	0	0	0	86

following 2 hr of incubation at room temperature the agglutination was evaluated in the usual fashion. Antiserum to whole human gamma globulin was prepared in this laboratory (1), whereas antisera to human IgG, IgM, and IgA were commercial preparations of Hyland Laboratories, Los Angeles, California.

Results. Agglutination of erythrocytes of foreign species origin. All Gambian sera were tested against erythrocytes of rhesus monkey, ox, sheep, guinea pig, and rat. The results of titrations were compared with similar titrations performed on sera from normal American population (2). No significant differences were found in titers of agglutinins for rhesus monkey, ox, or sheep erythrocytes. On the other hand, the Gambian population had significantly increased titers of antiguinea pig and antirat hemagglutinins. As shown in Table I, antiguinea pig hemagglutinin titers of 80 or higher were very seldom encountered in the normal American population and the same was true for titers of 160 or more of antirat hemagglutinin. In contrast, 22 Gambian sera had titers for guinea pig erythrocytes of 80 or more and 20 sera had antirat erythrocyte titers of 160 or more. As shown in Table II, there was a definite correlation between occurrence of these two hemagglutinins since 20 subjects had increased titers for both guinea pig and rat erythrocytes. On the other hand, appearance of these hemagglutinins was randomly distributed in subjects belonging to various ABO groups.

TABLE II. Antiguinea Pig and Antirat Hemagglutinins in Gambian Sera.

		Titer of guinea pig hemagglutinins		
		≤40	≥80	Total
Titer of rat hemagglutinins	≤80	16	2	18
	≥160	0	20	20
Total		16	22	38

Agglutination of A and B human erythrocytes. Table III presents the agglutination titers of Gambian sera observed in tests with human group A and group B erythrocytes. Blood groups of the serum donors were determined on the basis of slide tests performed with undiluted serum and standard blood group A and B erythrocyte suspensions. It is quite striking that subjects of blood group O had elevated titers of anti-A and anti-B agglutinins. Of 19 group O individuals there

TABLE III. Anti-A and Anti-B Titers of Gambian Sera.

19 Group O subjects		10 Group B subjects;	8 Group A subjects;
Anti-A	Anti-B	Anti-A	Anti-B
<10	<10	10	<10
20	20	10	<10
20	20	10	20
20	80	10	20
20	320	20	40
40	20	20	40
40	20	40	80
40	20	40	160
40	80	40	
80	20	40	
80	80		
160	80		
160	160		
160	160		
160	160		
160	320		
320	320		
320	320		
640	320		

TABLE IV. Effect of Absorption of Blood Group O Sera with Group A and B Erythrocytes.

Serum	Absorbed with	Titer of agglutination of erythrocytes group A	Titer of agglutination of erythrocytes group B
1	nil	320	320
	A	<10	40
	B	80	<10
2	nil	640	320
	A	<10	80
	B	80	<10

were 7 who showed increase in titers of both agglutinins to 160 or more. In addition, there was one subject who had a high titer of anti-B (320) with anti-A titer in a normal range (20), and another who had a high anti-A titer (160) with moderately increased anti-B titer (80). In rather sharp contrast, none of the individuals of blood group B and only one individual of blood group A had a hemagglutinin titer of 160.

This surprising occurrence of potent anti-A and anti-B agglutinins in blood group O sera stimulated an assumption that the reported findings are due to increased titer of the "cross-reacting," anti-A + B antibody, i.e., an antibody combining with both A and B erythrocytes (3–5). This assumption was supported by absorption experiments. Table IV shows results of a representative experiment in which two group O sera were examined. In both instances absorption with A erythrocytes not only removed the anti-A activity of the serum but also reduced its anti-B titer, whereas absorption with B erythrocytes not only abolished the anti-B activity but also reduced the anti-A titer.

Agglutination of trypsinized human erythrocytes. Eleven of 38 Gambian sera under investigation agglutinated trypsinized human erythrocytes of blood group O (Table V). Titers of 5–80 were observed. Controls included 35 sera of normal Americans and 154 sera of Americans suffering from various diseases. Sera of 3 patients produced weakly positive reactions, whereas the remaining control sera gave consistently negative results.

It was interesting to compare the reactions produced by the Gambian sera with reactions of antisera with known anti-Rh specificity. For this purpose, erythrocytes of various Rh genotypes were selected (Table VI). As expected, anti-Rh sera gave positive reactions only with those erythrocytes which contained the corresponding Rh antigens. In contrast, Gambian sera reacted equally well with all four Rh genotypes studied, which makes it highly unlikely that the activity of these sera was directed against any known Rh antigens. Moreover, the Gambian sera reacted equally well with the Le(a^-b^-) erythrocytes as with erythrocytes of other Lewis genotypes, which again indicates that the activity of these sera was not directed against any

TABLE VI. Agglutination of Trypsinized Blood Group O Erythrocytes by Gambian Sera and Rh Antisera.

Sera	Rh genotype CDe/Ce	cde/cde	cDE/cE	—D—
Gambian	+	+	+	+
Rh anti-CD	+	—	+	+
Rh anti-c	—	+	+	nd
Rh anti-E	—	—	+	nd
Rh anti-e	+	+	—	nd

TABLE V. Agglutination of Trypsinized Blood Group O Erythrocytes by Gambian and American Sera.

	No. of sera with a titer of <5	5	10	20	40	80	Total
Gambians	27	5	2	1	2	1	38
Normal Americans: Caucasians	24	0	0	0	0	0	24
Negroes	11	0	0	0	0	0	11
Americans with various diseases	151	3	0	0	0	0	154

TABLE VII. Reactions of Gambian Sera with Trypsinized Blood Group O Erythrocytes; Plain Agglutination (PA) and Indirect Coombs' Test with Anti-IgG (1), Anti-IgM (2) and Anti-IgA (3).

Serum dilution 1 to:	Serum A.J.				Serum A.B.			
		Coombs' Test				Coombs' Test		
	PA	1	2	3	PA	1	2	3
5	3+	nd	nd	nd	3+	nd	nd	nd
10	3+	nd	nd	nd	3+	nd	nd	nd
20	3+	nd	nd	nd	3+	nd	nd	nd
40	1+	1+	1+	3+	2+	nd	nd	nd
80	—	—	±	3+	1+	1+	1+	3+
160	—	—	—	2+	—	—	—	2+
320	—	—	—	1+	—	—	—	2+
640	—	—	—	—	—	—	—	1+

known Lewis antigens. On the other hand, none of the Gambian sera positive against trypsinized human erythrocytes agglutinated trypsinized erythrocytes of rhesus monkey.

We attempted to produce reactions between Gambian sera and unaltered human erythrocytes; we employed plain agglutination in saline, agglutination in serum and serum albumin medium, as well as indirect Coombs test with antisera to whole human γ-globulin and to various human immunoglobulin classes. All these tests gave consistently negative results.

Subsequently, indirect Coombs' tests were performed using Gambian sera and trypsinized human erythrocytes. Several sera were found to have considerably higher titers in the Coombs' test than in the plain agglutination test with trypsinized erythrocytes (see Table VII). It may be noted that this agglutination-enhancing effect could be produced by the anti-IgA but not by the anti-IgG or anti-IgM serum.

Because of these results, we reexamined, by indirect Coombs' test, 18 available Gambian sera which were previously negative in plain agglutination with trypsinized erythrocytes. This test was performed with trypsinized O erythrocytes using the anti-IgA reagent. Of 18 sera tested, 6 were positive with titers ranging from 20 to 80.

One positive Gambian serum could be tested against the individual's own erythrocytes which were suspended in glycerol and shipped on dry ice (6). Table VIII gives the results of titrations performed with two positive sera and two trypsinized erythrocyte samples. As shown serum A. J. gave equally strong reactions with erythrocytes of the unrelated donor and the individual's own erythrocytes. The second Gambian serum T.S., which was included as a control, also exhibited similar agglutination titers against both erythrocyte samples. The controls with untrypsinized erythrocytes were negative, indicating that the shipment of the erythrocytes did not increase their agglutinability.

Discussion. Agglutinins against guinea pig and rat erythrocytes in the Gambian sera may be considered "heterophile" antibodies that might have been formed in response to various infections. In this connection, observations of Houba *et al.* (7) are of interest, which showed increased titers of agglutinins to sheep erythrocytes as a result of infections with trypanosomata.

Increased titer of anti-A and anti-B hemagglutinins in sera of blood group O individuals is as interesting as it is enigmatic. One could hypothesize that these antibodies may also result from some infections but it is also possible that they were engendered by ingested antigens. From the absorption experiments it appeared likely that, at least in some sera under investigation, the titration for anti-A and anti-B activity actually measured cross-reacting anti-A + B antibody. Obviously, this was not the case in one group A individual with the anti-B titer of 160 or

TABLE VIII. Agglutination of Trypsinized Erythrocytes by the Individual's Own Serum.

Gambian sera	Trypsinized erythrocytes	Hemagglutination at serum dilution of 1 to: 5	10	20	40	80
A.J.	A.J.	3+	3+	3+	1+	—
	M.B.[a]	3+	3+	3+	2+	—
T.S.	A.J.	3+	3+	3+	2+	1+
	M.B.[a]	3+	3+	3+	2+	1+

[a] Unrelated blood group O donor.

in one group O individual who had anti-B titer as high as 320 but anti-A titer as low as 20.

Agglutinins with activity against trypsinized human erythrocytes, which were found in the Gambian sera, do not seem to be directed against antigenic sites of the erythrocyte surface, since negative results were obtained in all tests with untreated erythrocytes. These agglutinins are most likely directed against some antigen which is present in all human erythrocytes and which is exposed as a result of enzymatic digestion of erythrocytes. In these respects this antigen resembles the T antigen (8) which can be exposed on any human erythrocyte sample by digestion with filtrates of some bacteria.

The interesting question arose whether agglutinins to trypsinized erythrocytes have properties of iso- or autoantibodies. In one case tested, the Gambian serum acted upon the individual's own erythrocytes submitted to tryptic digestion suggesting the "auto" nature of these antibodies.

The physicochemical nature of the antibodies under investigation has not yet been explored; however, the results of the indirect Coombs' test strongly suggest that at least some portion of these antibodies belongs to the IgA class of immunoglobulins. In this connection, the paper by Adinolfi and his associates (9) is of some interest, since these authors observed sera with Rh antibodies of IgA nature which were detectable in the indirect Coombs' test.

Considering the origin of the hemagglutinin combining with trypsinized human erythrocytes, one is tempted to suggest that it is formed in response to excessive erythrocyte destruction which is associated with the exposure of normally hidden antigenic sites. Consistent with this hypothesis is the fact that all of the tested Gambians were exposed in their home environment to frequent infections with plasmodia of malaria.[1] Zuckerman (10) recently reviewed the evidence for formation of antibodies to erythrocytes in the course of malaria and the possible participation of such antibodies in the hemolytic anemia accompanying this disease. Since the agglutinins described in this report are not directed to cell surface antigens, they should be considered a sequel rather than the cause of the erythrocyte destruction.

Summary. Thirty-eight sera from Africans of Gambia were studied serologically. Twenty-two sera had increased titers of agglutinins to guinea pig erythrocytes and 20 had increased titers of rat hemagglutinins. Of 19 individuals of blood group O, 9 had increased titers of agglutinins to human A and/or B erythrocytes. Eleven of 38 sera agglutinated human blood group O erythrocytes exposed to trypsin. Possible relation of these serological findings to infectious processes, especially malaria, is discussed.

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[1] After completing this paper for publication, we received sera of 36 American volunteers with experimentally induced malaria. These were kindly supplied by Dr. P. G. Contacos, NIH Laboratory of Parasite Chemotherapy, Chamblee, Georgia. Of these sera, 6 had agglutinins acting upon trypsinized human erythrocytes, a finding strengthening the proposed hypothesis.

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