

INACTIVATION OF THE H RECEPTORS ON HUMAN ERYTHROCYTES BY AN ENZYME OBTAINED FROM *TRICHOMONAS FOETUS*.

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DURING studies designed to clarify the nature and properties of the serologically active receptors on human erythrocytes and in tissue fluids, attempts have been made to discover enzymes which could bring about the differential decomposition of the specific blood-group structures. Enzymes which decompose the specific water-soluble group materials have been described (Schiff, 1935, 1939; Schiff and Weiler, 1931*a, b*; Landsteiner and Chase, 1935; Iseki and Okada, 1951; Iseki and Tsunoda, 1952; Springer and György, 1953; Howe and Kabat, 1953) but the capacity of the enzymes to modify the receptors on the erythrocyte surface has not been extensively investigated. Attempts to destroy the group specificity of the intact erythrocyte by means of enzyme preparations obtained from *Clostridium welchii* were unsuccessful (Morgan, 1947) although the corresponding serologically active stromata, and the water-soluble A, B and H substances, were readily inactivated.

Watkins (1953) described an enzyme preparation extracted from the protozoon *Trichomonas foetus* which readily inactivated the water-soluble blood group substances. The present paper describes the action of a partially purified enzyme material obtained from *T. foetus* on certain of the specific blood group receptors on the red cell surface.

MATERIALS AND METHODS.

Source of enzyme.—The enzyme was obtained from living *T. foetus* organisms as described by Watkins (1953). The crude extract was fractionated once from aqueous solution with ammonium sulphate and the fraction precipitated at 2° between the levels 24–30 per cent w/v ammonium sulphate concentration was collected, dialysed to remove salts and dried from the frozen state. This material destroyed the activity of water-soluble preparations of human H substance but was without action on preparations of human A and B substances. The H-destroying activity of the enzyme preparation expressed in terms of the unit defined by Stack and Morgan (1949) was 114 units per mg. nitrogen.

Treatment of red cells with the enzyme.—Suspensions (1 per cent) in saline (1 ml.) of red cells of known phenotype were treated with 1 mg. of enzyme for 2 hr. at 37°. The suspension was shaken gently at frequent intervals during the incubation period and the red cells were then collected, washed three times and re-suspended in saline. Suspensions of cells after treatment with the enzyme were stable and were free from haemolysis.

Agglutination tests.—These were carried out as described previously (Morgan and Watkins, 1951).

Anti-H sera.—Human and animal sera which agglutinate O and A₂ cells more strongly than cells of other groups can be divided into two groups: those which are inhibited by saliva from secretor persons belonging to all the ABO groups are called anti-H sera and those

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which are not inhibited are at present grouped together as anti-O sera (Morgan and Watkins, 1948). Three examples of anti-H sera of different origin were used in the present investigation: the naturally occurring anti-H agglutinin in the serum of the eel *Anguilla anguilla* (Sugishita, 1935; Jonsson, 1944), an immune anti-H serum produced by injection of rabbits with human H substance isolated from ovarian cyst fluids (Morgan and Waddell, 1945) and a powerful human anti-H serum "Tomlinson," which acted on O cells to a titre of 32,000 at 21°. The action of the *T. foetus* enzyme on those receptors detected by the O cell agglutinins which are not anti-H in character has not been studied.

RESULTS.

The Action of T. foetus Enzyme on the Specific Blood Group Receptors on the Erythrocyte Surface.

The H receptors.—The results of the titration of two samples of group O cells, before and after treatment for 2 hr. at 37° with the *T. foetus* enzyme, against the human anti-H serum "Tomlinson" and natural eel serum are shown in Table I. The agglutination results recorded were read after the tests had been allowed to stand for 2 hr. at 21°. The marked fall in titre after incubation of both samples of group O cells with the enzyme indicates that the H receptors on the cell surface have been rendered serologically inactive by this treatment. Absorption experiments with the cells after treatment with enzyme confirmed these results. The human serum "Tomlinson" showed only a slight reduction in titre against untreated O cells after two absorptions with enzyme-treated O cells whereas when the absorptions were carried out with the same volume of untreated cells the anti-H agglutinins were almost completely removed.

The residual agglutination titre given by the enzyme-treated cells with the anti-H sera (Table I) was not diminished after more prolonged treatment of the cells with the *T. foetus* preparation; this fact, combined with the observation that the treated cells were agglutinable to a similar extent by an anti-A serum which, of course, failed to react with the untreated O cells, suggested that the residual titre was not due to unchanged H receptors on the cell surface but was the result of some modification of the cells which rendered them non-specifically agglutinable by serum. This suggestion was confirmed by inhibition tests with human H substance which failed to block the residual agglutination of enzyme-treated cells by the anti-H sera. Subsequent experiments showed that when human sera were used as the test reagents, the non-specific agglutination could be confined to the first two or three dilutions only, if the tests were read after 2 hr. at 37°. The rabbit anti-H serum, however, reacted so strongly with enzyme-treated cells of all groups, even at 37°, that it was not possible to determine whether the H receptors detected by this serum were destroyed by the enzyme. The possibility of removal of the non-specific agglutinin by absorption was therefore investigated. A specimen of group A₁ cells, which reacted very weakly with the rabbit anti-H serum at 21°, was treated with the *T. foetus* enzyme preparation, the cells were washed three times with saline and the rabbit anti-H serum was then absorbed twice with one quarter volume of the enzyme-treated cells. Titration of the absorbed serum against treated and untreated O cells showed that (a) the non-specific agglutinins could be removed by absorption (b) absorption did not impair the capacity of the rabbit serum to react with untreated O cells and (c) the H activity of the O cells detected by the rabbit anti-H serum was destroyed by treatment with the enzyme.

TABLE I.—Action of *T. foetus* Enzyme on H receptors of Group O Erythrocytes.

Serum.	Cells.			Dilution of serum.														
	Donor.	Treatment.	Group.	1:2.	1:4.	1:8.	1:16.	1:32.	1:64.	1:128.	1:256.	1:512.	1:1024.	1:2048.	1:4096.	1:8192.	1:16384.	1:32768.
Human anti-H "Tomlinson".	W. T. J. M.	Untreated.	A ₁	4	4	4	4	4	4	4	4	3	3	3	3	2	2	1
		Enzyme-treated.	A ₁	3	3	3	2	1	0	0	0	0	0	0	0	0	0	0
Natural eel serum.	G. S.	Untreated.	"	4	4	4	3	3	3	3	3	3	3	3	2	2	1	0
		Enzyme-treated.	"	3	2	2	1	0	0	0	0	0	0	0	0	0	0	0
	W. T. J. M.	Untreated.	"	3	3	3	3	3	3	3	2	2	1	0	0	0	0	0
		Enzyme-treated.	"	3	3	2	1	0	0	0	0	0	0	0	0	0	0	0
	G. S.	Untreated.	"	3	3	3	3	3	3	2	2	1	0	0	0	0	0	0
		Enzyme-treated.	"	3	3	2	1	0	0	0	0	0	0	0	0	0	0	0

Degrees of agglutination: 0, absence of agglutination; 1, groups of 2-3 cells; 2, larger groups with many free cells; 3, agglutination visible to the naked eye; 4, massive agglutination.

TABLE II.—Action of *T. foetus* Enzyme on A, B and H Receptors of A₁ and B Cells.

Serum.	Cells.			Dilution of serum.											
	Donor.	Treatment.	Group.	1:2.	1:4.	1:8.	1:16.	1:32.	1:64.	1:128.	1:256.	1:512.	1:1024.	1:2048.	1:4096.
Human anti-H "Tomlinson"	E. I.	Untreated.	A ₁	3	3	3	3	3	3	3	2	2	1	0	0
		Enzyme-treated.	A ₁	1	0	0	0	0	0	0	0	0	0	0	0
Immune human anti-A "McCrae"	"	Untreated.	"	4	4	4	4	4	4	3	3	3	2	1	0
		Enzyme-treated.	"	4	4	4	4	4	4	3	3	3	2	1	0
Human anti-H "Tomlinson"	P. P.	Untreated.	A ₁	0	0	0	0	0	0	0	0	0	0	0	0
		Enzyme-treated.	A ₁	1	0	0	0	0	0	0	0	0	0	0	0
Immune human anti-A "McCrae"	"	Untreated.	"	4	4	4	4	4	4	3	3	3	2	1	0
		Enzyme-treated.	"	4	4	4	4	4	4	3	3	3	2	1	0
Human anti-H "Tomlinson"	D. P.	Untreated.	B	3	3	3	3	3	3	2	2	1	0	0	0
		Enzyme-treated.	B	1	0	0	0	0	0	0	0	0	0	0	0
Immune human anti-B "Clark"	"	Untreated.	"	4	4	4	3	3	3	3	3	3	2	1	0
		Enzyme-treated.	"	4	4	4	3	3	3	3	3	3	2	1	0

* Anti-B agglutinin neutralised with human B substance. Degrees of agglutination as in Table I.

As a result of these observations all the following experiments involving titration of enzyme-treated cells against the homologous serum were carried out either at 37° or with sera which had previously been absorbed with enzyme-treated cells which did not contain the blood group receptor under investigation.

The A₁, A₂ and B receptors.—The *T. foetus* enzyme preparation used for the above experiments was without action on the water-soluble A and B substances and, therefore, it was of interest to determine whether the enzyme preparation would exhibit the same selectivity towards the receptors on the erythrocyte surface. The agglutination results obtained after treatment of A₁ and B cells with the enzyme and subsequent incubation of the cells and serum for 2 hr. at 37°, are given in Table II. The serum "Tomlinson" contained an anti-B agglutinin which was neutralised before titration of the serum against B cells with a human B substance, which contained no significant amount of H activity. Two A₁ donors, one (E. I.) whose cells reacted strongly with the human anti-H serum "Tomlinson" and a second (P. P.) whose cells failed to react at 37° with this reagent, were included in the experiment. The H activity of the strongly reacting A₁ cells and that of the B cells was destroyed, but the respective A and B activities of these cells as measured by immune human anti-A and anti-B sera, were unchanged. Treatment of A₂ cells with the *T. foetus* preparation resulted in a similar loss of H activity without impairment of the cells' capacity to react with anti-A sera.

The Rhesus (D) receptors.—Rh agglutination tests are regularly carried out at 37° and therefore it was possible to determine the action of the enzyme on the Rh(D) factor without previous absorption of the test sera. Table III shows the results of the titration of enzyme-treated R₁R₁ and rr cells against a saline-agglutinating anti-D and an incomplete anti-D serum. The activity of the R₁R₁ cells against the saline-agglutinating anti-D was not reduced and a capacity to react with the incomplete anti-D serum was developed. The *T. foetus* enzyme, therefore, does not destroy the Rh(D) receptors and the reactivity of the enzyme-treated cells in saline with incomplete anti-D serum suggests that the enzyme preparation brings about a modification of the cell surface similar to that effected by trypsin or papain treatment.

TABLE III.—Action of *T. foetus* Enzyme on Rhesus D Receptor.

Serum.	Cells.			Dilution of serum.							
	Donor.	Group.	Treatment.	1:2.	1:4.	1:8.	1:16.	1:32.	1:64.	1:128.	1:256.
Saline anti-D	W. T. J. M.	R ₁ R ₁	Untreated .	3	3	3	3	3	2	1	0
			Enzyme-treated	3	3	3	3	3	2	1	0
	D. P.	rr	Untreated .	0	0	0	0	0	0	0	0
			Enzyme-treated	1	0	0	0	0	0	0	0
Incomplete anti-D	W. T. J. M.	R ₁ R ₁	Untreated .	0	0	0	0	0	0	0	0
			Enzyme-treated	3	3	3	3	3	2	2	1
	D. P.	rr	Untreated .	0	0	0	0	0	0	0	0
			Enzyme-treated	0	0	0	0	0	0	0	0

Degrees of agglutination as in Table I.

The M, N and S receptors.—Modification of the M and N receptors on the cell surface by treatment with enzymes has been previously reported (Morton and

Pickles, 1951; Rosenfield and Vogel, 1951; Puschel, 1953). The *T. foetus* enzyme preparation was also found to render the M and N receptors serologically inactive (Table IV). The immune rabbit anti-M and anti-N sera were absorbed with enzyme-treated N and M cells respectively and titrations against the homologous enzyme-treated cells were then carried out at room temperature. Tests for the S factor were made with an unabsorbed human anti-S serum at 37°; there was no evidence that the S receptors had been modified by treatment with the *T. foetus* enzyme.

TABLE IV.—Action of *T. foetus* Enzyme on MNS Receptors.

Serum.	Cells.		Dilution of serum.					
	Group.	Treatment.	1:2.	1:4.	1:8.	1:16.	1:32.	1:64.
Rabbit anti-M (absorbed with enzyme-treated N cells)	MM	Untreated .	3	3	3	2	1	0
		Enzyme-treated .	0	0	0	0	0	0
	NN	Untreated .	0	0	0	0	0	0
		Enzyme-treated .	0	0	0	0	0	0
Rabbit anti-N (absorbed with enzyme-treated M cells)	NN	Untreated .	3	2	1	0	0	0
		Enzyme-treated .	0	0	0	0	0	0
	MM	Untreated .	0	0	0	0	0	0
		Enzyme-treated .	0	0	0	0	0	0
Human anti-S (unabsorbed)	SS	Untreated .	3	2	2	1	0	0
		Enzyme-treated .	3	3	2	1	0	0
	ss	Untreated .	0	0	0	0	0	0
		Enzyme-treated .	?	0	0	0	0	0

Degrees of agglutination as in Table I.

The P receptors.—Treatment of P-positive cells with the *T. foetus* enzyme preparation did not result in loss of activity when the cells were titrated against a human anti-P serum which had previously been absorbed with enzyme-treated P-negative cells.

The Lewis Le^a and Le^b receptors.—Experiments carried out to determine whether the Le^a and Le^b receptors on the cell surface are modified by the *T. foetus* enzyme suggest that inactivation does not occur. It was not possible to establish conclusively that these structures were unchanged because the anti-Le^a and anti-Le^b sera available were of such low titre and avidity.

The Action of Trypsin and Papain in the H Receptors on the Erythrocyte Surface.

Rosenfield and Vogel (1951) reported that the O(H) factor is not essentially changed by trypsin treatment as measured by either eel serum or human serum as the source of anti-O (H). The action of the *T. foetus* preparation on the Rh(D) receptors and the MNS receptors, however, so closely paralleled the results obtained

with trypsin-treated cells that it was thought desirable to confirm that the proteolytic enzymes used for Rh sensitisation did not destroy the activity of the O cells against the anti-H reagents which were used to test the *T. foetus* enzyme-treated cells. A sample of Difco trypsin, which was known to cause enhancement of Rh agglutination and destruction of the M and N receptors was kindly supplied by Miss Joan Thompson. This preparation did not cause any fall in the agglutination titre of the cells against the human anti-H serum "Tomlinson." Similarly treatment of cells with papain by the method described by Stratton (1953) failed to cause any diminution in H activity.

Treatment of human erythrocytes with periodate destroys the specific blood-group receptors on the cell surface (Moskowitz and Treffers, 1950; Morgan and Watkins, 1951). Recently, Coffin and Pickles (1953) have shown that subsequent treatment with trypsin restores the specific agglutinating capacity of erythrocytes which have been treated with periodate. These authors suggested that the action of the trypsin was to unmask previously unavailable antigen sites on the erythrocyte surface and it therefore became of interest to determine whether further H receptors would be revealed by the action of trypsin on group O cells which had lost their reactivity with anti-H sera following their exposure to the *T. foetus* enzyme. Trypsin did not, however, restore the agglutinating capacity of the *T. foetus* enzyme-treated O cells against the human anti-H serum "Tomlinson."

The Action of the T. foetus Enzyme on the Virus Receptors on the Erythrocyte Surface.

An examination of the behaviour in the virus haemagglutination test of group O cells which had been treated with *T. foetus* enzyme and the preparation of cells treated with living influenza virus were kindly undertaken by Professor Wilson Smith. The *T. foetus* enzyme-treated cells were found to be unsusceptible to haemagglutination with the PR8 strain of influenza virus. The results of a preliminary adsorption and elution experiment indicated that the treated cells fail to adsorb the virus and this observation supports the conclusions drawn from the haemagglutination test that the virus receptors on the red cell surface are destroyed by the *T. foetus* enzyme.

Normal human group O cells which had been treated with living influenza virus were found to be non-specifically agglutinated by human serum and could not, therefore, be used to test for the presence of H receptors by the usual agglutination procedure. The virus-treated cells were, however, as effective in absorbing the anti-H agglutinins from the human anti-H serum "Tomlinson" as were the untreated O cells. It would, therefore, appear that exposure of the cells to living virus, under conditions which destroy the virus receptors, does not result in the destruction of the H receptors on the cell surface.

The Inhibition of the Action of the T. foetus Enzyme on the H and M Receptors on the Erythrocyte Surface.

Incubation of L-fucose with the substrate-enzyme mixture inhibits the action of the *T. foetus* enzyme preparation on the water-soluble H substance (Morgan and Watkins, unpublished observations); experiments were therefore carried out to determine whether this sugar would have the same inhibitory effect on

the destruction of the H receptors on the red cell surface. Cell suspensions were prepared in 1 per cent solutions of fucose dissolved in saline and were then incubated with the *T. foetus* enzyme for 2 hr. Titration of the enzyme-treated cell suspensions against the human anti-H serum "Tomlinson" at 37° revealed that the presence of 10 mg. L-fucose inhibited completely the action of 1 mg. of the enzyme preparation on the H receptors contained in 1 ml. of a 1 per cent O cell suspension. The presence of this quantity of fucose did not, however, inhibit the action of the *T. foetus* preparation on the M and N receptors of the O cells.

The carbohydrate portion of the mucoid showing H specificity contains four sugars in about equal amounts, L-fucose, D-galactose, D-glucosamine and D-galactosamine. The inhibitory effect of L-fucose led us to test D-fucose, and also the other constituent sugars of the H substance, for their capacity to inhibit the destruction of the H receptors by the *T. foetus* enzyme. D-galactosamine, used at a concentration of 10 mg. of the sugar to 1 mg. of enzyme, was found to be as effective as L-fucose in preventing the inactivation of the H receptors, whereas D-galactose and D-fucose at the same concentration had only a slight inhibitory action and D-glucosamine was completely inactive as an inhibitor. None of these sugars showed any capacity to inhibit the destruction of the M receptors by the *T. foetus* enzyme.

The water-soluble human blood group substances were also tested for their power to inhibit the action of the *T. foetus* enzyme on the H and M receptors on the cell surface. A, B and Le^a substances at a concentration of 1.5 per cent failed to inhibit the action of 1 mg. of enzyme on the H receptors in 1 ml. of a 1 per cent suspension of group O cells. H substance at the same concentration partially inhibited the destruction of the H receptors. All four blood group substances, however, completely inhibited the action of the *T. foetus* enzyme on the M receptors.

Human serum was found to inhibit the destruction of both the H and M receptors by the *T. foetus* enzyme. The presence of 0.02 ml. human group A serum in a final volume of 1 ml. was sufficient to cause partial inhibition of the action of 1 mg. of the enzyme preparation on 1 per cent group OM cells; 0.10 ml. serum gave almost complete inhibition.

DISCUSSION.

The serological properties of the intact human erythrocyte have been examined extensively but comparatively little is known about the chemical nature of the constituents of the erythrocyte membrane which participate in the serological reactions. At least nine independent blood group systems involving some thirty serologically distinct antigens have been identified on the erythrocyte surface by means of the corresponding antibodies (Race and Sanger, 1950); other receptor sites have been revealed by studies on virus haemagglutination (Hirst, 1942; Burnet, 1952). Each group-specific material occurs on the erythrocyte surface in very small amounts, however, and this fact, and the firm combination which seems to exist between these materials and lipid or lipoprotein constituents of the cell membrane, make difficult their isolation in quantities sufficient for detailed chemical studies. Consequently, our present knowledge of the chemical and physical properties of the human blood group substances has been obtained

largely through a study of the water-soluble materials which show specificities corresponding to certain of the erythrocyte blood group characters and which occur in the tissue fluids and secretions of the majority of individuals. The water-soluble materials responsible for the A, B, H and Le^a specificities have each been isolated in an essentially homogeneous condition and identified as mucoids. Materials showing blood group specificity have been obtained in small quantities from erythrocytes by extraction with ethanol (Hallauer, 1934 ; Stepanov, Kuzin, Markageva and Kosjakov, 1940 ; Rasch, 1952) and the chemical analyses of the relatively crude materials obtained have not been inconsistent with the attribution of a mucoid nature to these group-specific complexes. It has yet to be determined whether the specifically reactive structures on the erythrocyte surface are chemically identical with those responsible for the blood group character of the water-soluble materials.

An attack on the red cell surface by means of enzymes of known specificity presents an indirect method for obtaining evidence as to the nature of specific receptor sites. Morton and Pickles (1951), in an investigation on the proteolytic enzyme test for the detection of incomplete antibodies, discovered that trypsin will destroy the structures responsible for the serological M and N characters ; this was confirmed by Rosenfield and Vogel (1951). The inactivation of the M and N receptors by a proteolytic enzyme preparation suggests that the materials associated with these two specificities might be protein in nature but does not give any precise information as to the structures modified by the enzyme action. Enzymes which destroy the serological activity of the water-soluble blood group substances have been isolated from several different sources but, except for the blood group enzyme from *Cl. welchii*, which failed to bring about inactivation of the blood group receptors on the intact erythrocyte (Morgan, 1947), it is not known whether these enzymes behave towards the receptors on the red cell surface as they do towards the materials of similar specificity in solution. The main difficulty associated with the examination of the action of the blood group-destroying enzymes on the red cells arises from the fact that the enzyme preparations used are frequently accompanied by other substances or enzymes which cause either haemolysis of the red cells or the development of extensive non-specific agglutination. The partially purified enzyme preparation derived from *T. foetus*, which was used for the experiments described here, did not cause haemolysis, and stable unagglutinated suspensions of the treated cells could be prepared in saline. At room temperature the treated cells were to some extent agglutinated non-specifically in serum but this difficulty was largely overcome by carrying out the agglutination tests at 37° or by previous absorption of the sera with *T. foetus*-treated cells which did not contain the receptor under investigation. The *T. foetus* enzyme preparation had been shown (Watkins, 1953) to destroy the serological specificity of the water-soluble H substance, but to be without action on the water-soluble materials associated with A, B and Le^a specificities. The observation that when the *T. foetus* enzyme is allowed to act on intact red cells the H receptors are rendered serologically inactive whereas the A and B structures are not detectably modified suggests that the same enzyme is responsible for the inactivation of the H substance in solution and on the red cell surface. The inhibition of the action of the *T. foetus* preparation on both the water-soluble H material and the H receptors on the intact erythrocyte by L-fucose and D-galactosamine gives further support for the view that the same enzyme is involved in

the destruction of both forms of H specific structures. The partial inhibition of the enzymic destruction of the H receptors on the red cell surface by a solution of H substance and the failure of the A, B and Le^a substances to cause similar inhibition is evidence that in the former instance the enzyme is competing for the same substrate. These results can therefore be taken as an indication that the serologically specific structures of the H substance in solution and the H receptors on the red cell surface are chemically similar.

The *T. foetus* enzyme preparation, in addition to its action on the H receptors, causes other modifications in the behaviour of the erythrocyte. Thus, group M and N cells lose the capacity to react with their homologous antibodies, Rh-positive cells become agglutinable in saline by incomplete anti-D sera, and the receptors on the cell surface for the PR8 strain of influenza virus are destroyed. The failure of L-fucose and D-galactosamine to prevent the destruction of the M and N serological activities, and the inhibition of this destruction by all the water-soluble blood group complexes tested, suggests that the enzyme responsible for the destruction of the M and N receptors is different from the H-inactivating enzyme. The development of agglutinability in saline with incomplete anti-D sera following the treatment of Rh-positive cells with the *T. foetus* enzyme, and the destruction of the M and N factors without loss of activity of the closely related S factor parallels very closely the effect of treating cells with trypsin as described by Rosenfield and Vogel (1951). Trypsin, however, is without action on the H receptors of the red cell and the results would suggest that the *T. foetus* preparation contains in addition to a specific enzyme which inactivates H receptors, other enzymes which are similar in their properties to those present in the proteolytic enzyme preparations used for Rh sensitisation. Similarly, although treatment of red cells with the *T. foetus* preparation inactivates the virus receptors, destruction of these receptors by exposure of the cells to living virus does not result in loss of H activity. The virus receptor-destroying enzyme in the *T. foetus* preparation would again appear to be a different enzyme from the H-inactivating enzyme.

Coffin and Pickles (1953) showed that red cells which had been inactivated serologically by treatment with periodate could be rendered agglutinable again for certain serological characters by treatment with trypsin. They considered that the cell surface was disrupted by the action of trypsin and that further material of similar specificity was exposed. In view of the observation that the *T. foetus* preparation contains an enzyme similar in its properties to trypsin it is not surprising that in our experiments the treatment of red cells with trypsin after they had been exposed to the action of *T. foetus* enzyme failed to reveal additional H receptors.

The earlier results on the action of viruses and proteolytic enzymes, together with those of the experiments described here, show that specific and controlled changes of the serologically reactive sites on the erythrocyte surface can be brought about by enzyme action. It may be possible, therefore, by the action of carefully chosen and purified enzymes to obtain red cells devoid of certain predetermined serologically reactive structures and thus to allow many absorption, agglutination and immunisation experiments to be performed which could not be carried out with unchanged cells. Similarly, it should be possible to determine the influence of individual immunologically specific receptors on the physical behaviour of the erythrocyte. A knowledge of these properties of erythrocytes should help to

clarify our understanding of the basic chemical and physical reactions responsible for their observed behaviour in the fields of virology and blood group serology.

SUMMARY.

The action of a partially purified enzyme preparation obtained from *Trichomonas foetus* on certain of the specific blood group receptors on the erythrocyte surface is described.

This enzyme inactivates the H-specific structures and those responsible for M and N specificity but is without action on the A, B, P or S receptors. Treatment of Rh(D)-positive cells leads to the development of specific agglutinability in saline with incomplete anti-D serum.

The enzymes responsible for the inactivation of the H and M receptors can be differentiated by inhibition tests. The destruction of the H character is inhibited by L-fucose and D-galactosamine whereas these sugars do not inhibit the inactivation of the M receptors.

Erythrocytes which have had their H receptors destroyed by the *T. foetus* enzyme are no longer agglutinated by the PR8 strain of influenza virus. Their treatment with the same virus does not bring about the destruction of the H-specific structures.

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REFERENCES.

- BURNET, F. M.—(1952) *Annu. Rev. Microbiol.*, **6**, 229.
 COFFIN, S. F., AND PICKLES, M. M.—(1953) *J. Immunol.*, **71**, 177.
 HALLAUER, C.—(1934) *Z. ImmunForsch.*, **83**, 114.
 HIRST, G. K.—(1942) *J. exp. Med.*, **75**, 49.
 HOWE, C., AND KABAT, E. A.—(1953) *J. Amer. chem. Soc.*, **75**, 5542.
 ISEKI, S., AND OKADA, S.—(1951) *Proc. Acad. Japan*, **27**, 455.
Idem AND TSUNODA, S.—(1952) *Ibid.*, **29**, 370.
 JONSSON, B.—(1944) *Acta path. microbiol. scand.*, Suppl., **54**, 456.
 LANDSTEINER, K., AND CHASE, M. W.—(1935) *Proc. Soc. exp. Biol., N.Y.*, **32**, 713.
 MORGAN, W. T. J.—(1947) *Experientia*, **3**, 257.
Idem AND WADDELL, M. B. R.—(1945) *Brit. J. exp. Path.*, **26**, 387.
Idem AND WATKINS, WINIFRED M.—(1948) *Ibid.*, **29**, 159.—(1951) *Ibid.*, **26**, 387.
 MORTON, J. A., AND PICKLES, M. M.—(1951) *J. clin. Path.*, **4**, 189.
 MOSKOWITZ, M., AND TREFFERS, H. P.—(1950) *Science*, **111**, 717.
 PUSCHEL, J.—(1953) *Z. Hyg. InfektKr.*, **137**, 67.
 RACE, R. R., AND SANGER, R.—(1950) 'Blood Groups in Man.' Oxford (Blackwell).
 RASCH, L.—(1952) *Z. ImmunForsch.*, **110**, 243.
 ROSENFELD, R. E., AND VOGEL, P.—(1951) *Trans. N.Y. Acad. Sci.*, **13**, 213.
 SCHIFF, F.—(1935) *Klin. Wschr.*, **14**, 750.—(1939) *J. infect. Dis.*, **65**, 127.
Idem AND WEILER, G.—(1931a) *Biochem. Z.*, **235**, 454.—(1931b) *Ibid.*, **239**, 489.
 SPRINGER, G. F., AND GYÖRGY, P.—(1953) *Fed. Proc.*, **12**, 272.
 STACK, M. V., AND MORGAN, W. T. J.—(1949) *Brit. J. exp. Path.*, **30**, 470.
 STEPANOV, A., KUZIN, A., MARKAGEVA, Z., AND KOSJAKOV, P.—(1940) *Biochimia*, **5**, 547.
 STRATTON, F.—(1953) *Vox Sanguinis*, **3**, 43.
 SUGISHITA, S.—(1935) *J. Jizen med. Soc.*, **40**, 1938.
 WATKINS, WINIFRED M.—(1953) *Biochem. J.*, **54**, xxxiii.