OXYGEN-STABLE HEMOLYSINS OF GROUP A STREPTOCOCCI

III. The Relationship of the Cell-Bound Hemolysin to Streptolysin S*

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It has been previously shown (1) that various strains of Group A streptococci possess a cell-bound hemolytic factor (CBH) which can be demonstrated by the incubation of red blood cells (RBC) of various animal species with washed streptococci, in the presence of glucose, Mg^{++}, and sulfhydryl compounds. Under such conditions, hemolysis occurs within a short time, although no extracellular hemolysin can be demonstrated in supernates of the incubation mixture or in extracts of streptococci disrupted by sonic oscillation. Also, washed streptococci with CBH activity have been found to cause distinct cytopathogenic changes in Ehrlich ascites tumor cells and in a variety of other mammalian cells *in vitro* (2, 3). The hemolytic as well as the cytopathogenic effect caused by the washed streptococci can be abolished by a variety of metabolic inhibitors and by factors known to inhibit streptolysin S activity (lecithin, trypan blue) (1). The possible relationship of the CBH to streptolysin S, that is, the group of oxygen-stable streptococcal hemolysins, was briefly discussed previously (1). It was found that only streptococcal strains producing streptolysin S possess CBH activity, and that inhibition of CBH activity also results in the inhibition of streptolysin S formation. However, the nature of the CBH, its possible relationship to streptolysin S, and the mechanism of its lysis of RBC in the absence of a cell-free hemolysin remained obscure.

In a recent report (4) it was shown that the oxygen-stable hemolysins of Group A streptococci comprised a group of substances with a given hemolytic moiety which can be bound to any of a diverse group of carrier molecules (RNA, serum albumin, serum α-lipoprotein, tween, and triton) and that the hemolytic moiety can be transferred from one carrier to another, in certain directions, within the group. Also, it was shown that the degree of affinity for the hemolytic group differed among the

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carriers. It was suggested that the prosthetic group of the CBH and the oxygen-stable hemolysins might be the same, the carriers of the oxygen-stable hemolysins having greater affinity for the hemolytic moiety than does the streptococcal component in its original cell-bound form, so that these molecular species, if present in streptococcal suspensions, become recognized as inducers of oxygen-stable hemolysins. Finally it was suggested that some cell membrane component of the RBC and other cells have still higher affinity for the hemolytic group than any of the carrier molecules, so that they are affected by either CBH or oxygen-stable hemolysins.

In the present paper, a further study on the CBH and its relationship to streptolysin S will be reported. Studies on the possible mechanism of lysis of RBC by the CBH will be presented in the following paper.

Materials and Methods

Culture Media were described in a preceding paper (5).

Streptococcal Strains.—Most of the experiments described below were performed with strain S84, type 3, obtained from the State Serum Institute in Copenhagen. In a few experiments, strains C203S, C203U (type 3), and strain Blackmore (type II), which were supplied by Dr. A. W. Bernheimer of the New York University School of Medicine, and strain Richard, type 3, and a glossy type 6 (obtained from the National College of Type Cultures, London) were also employed. The streptoocci were cultivated in 200-ml volumes of brain heart infusion (BHI), Difco Laboratories, Inc., Detroit. After the cultures had reached the stationary phase of growth, the cells were harvested, washed with 200 ml of saline, buffered with 0.05 M phosphate at pH 7.4, and resuspended in 10 ml of the buffer. Of such cell suspensions 0.2-ml amounts were employed in all experiments. The optical density of the streptococcal growth was checked with a spectrophotometer at 550 mμ.

Preparation and Partial Purification of the Hemolysins.—

1. Preparation of RNA hemolysin: The preparation of an active fraction of RNA core by ion exchange chromatography was described in detail in a previous publication (4). Streptococci obtained from a 200 ml culture in brain heart infusion broth were harvested from the stationary phase of growth, washed, and incubated in 20 ml, with maltose, $MgSO_4 \cdot 7H_2O$, and cysteine, each at 0.001 M, and RNA core fraction at 1 mg/ml. After incubation at 37°C for 30 minutes the hemolysin-containing supernate which had 10 to 15 $\times$ 10^3 hemolytic units per ml, was chilled to 0°C and 8 volumes of methanol at −20°C were added. The mixture was kept at 0°C for 15 to 20 minutes. A flaky precipitate was removed by centrifugation at 3000 RPM for 5 minutes and was found on resolution to contain 90 to 100 per cent of the total hemolytic activity. The precipitate was dissolved in 1 ml of buffered saline solution and the slightly opalescent solution, containing about 250,000 H.U./ml, was applied to a 1 x 3 cm column of DEAE-sephadex A-50 previously washed with ice cold buffered saline. Potassium chloride was added to the buffer in the column at 0.3 M and 1.0 M in two steps; approximately 75 per cent of the hemolytic activity was recovered in the 1 M KCl effluent of the column. The hemolysin was precipitated once more with 8 volumes of methanol at −20°C and was stored at −20°C, preferably as a pellet.

2. Cell-bound hemolysin: Washed streptococci were serially diluted with the buffer solution indicated above in a volume of 0.3 ml. To each tube were added maltose (0.005 M), $MgSO_4 \cdot 7H_2O$ (0.001 M), and cysteine (0.001 M); these three components will be referred to as the activation mixture (AM). The tubes were incubated for 10 minutes at 37°C, after which 0.5 ml of 2 per cent human RBC was added and the incubation mixture was made up to 1.0 ml with buffer. The tubes were incubated for 30 minutes at 37°C and centrifuged and the degree

of hemolysis was determined with the spectrophotometer at 550 mμ. One hemolytic unit (H.U.) of CBH was defined as the amount of a streptococcal suspension (5×10^8 cells per ml) which hemolyzes 50 per cent of a 2 per cent human RBC suspension (final concentration 1 per cent) after 30 minutes of incubation at 37°C. To assure that streptolysin O was not the cause of any hemolysis observed, 25 μg/ml of cholesterol were included in all reaction mixtures (6).

Unitage of CBH and RNA Hemolysins.—Because CBH can be detected only in the presence of streptococci, which can continue to produce hemolysins while the hemolytic effect is being measured, no attempt is made to compare the unitage of CBH with that of the RNA core hemolysin. Only internal comparisons within each system are given.

RESULTS

A. Attempts to Detect a Cell-Free Hemolysin in Streptococcal Suspensions Possessing CBH Activity

As demonstrated previously (1) no extracellular hemolysin could be demonstrated in supernates of washed streptococci (strain S84) possessing high CBH activity. In the present study further attempts to detect hemolytic activity in the suspending medium of such streptococci were made by increasing the sensitivity of the hemolytic test. In some experiments, the concentration of RBC was reduced to a 0.1 per cent suspension, which was found, in estimates of RNA hemolysin, to increase the sensitivity of the assay by approximately 5-fold. In other experiments a hypotonic medium was used, approaching the lower osmotic limit consistent with the maintenance of human RBC, which had been found to produce a further 3-fold increase in sensitivity. In order to assure that no streptococci were present in such supernates they were first passed through a

TABLE I

Role of Maltose, Mg^{++}, and Cysteine in the Production of Cell-Bound Hemolysin and RNA-Induced Hemolysin

Included in incubation mixture			Hemolytic activity	
Maltose	Mg^{++}	Cysteine	CBH	RNA hemolysin*
			units/ml	*units/ml*
−	−	−	30	0
+	−	−	35	5
−	+	−	40	0
−	−	+	150	150
+	+	−	650	500
+	−	+	1250	2250
−	+	+	2560	650
+	+	+	3000	2500

* 100 μg/ml of RNA-resistant core added to all tubes. Maltose, cysteine, Mg^{++} at final concentrations as indicated in Materials and Methods.

Millipore filter (0.22 μ). (Under similar conditions a solution of RNA hemolysin possessing 10 H.U./ml was totally recovered following filtration through a millipore filter.) In no case was hemolytic activity detected in supernates of the active streptococcal suspensions.

B. The Relationship of the Cell-Bound Hemolysin to Streptolysin S

As previously suggested, the cell-bound hemolysin of streptococci may represent a hemolytic moiety synthesized by streptococci originally bound to a com-

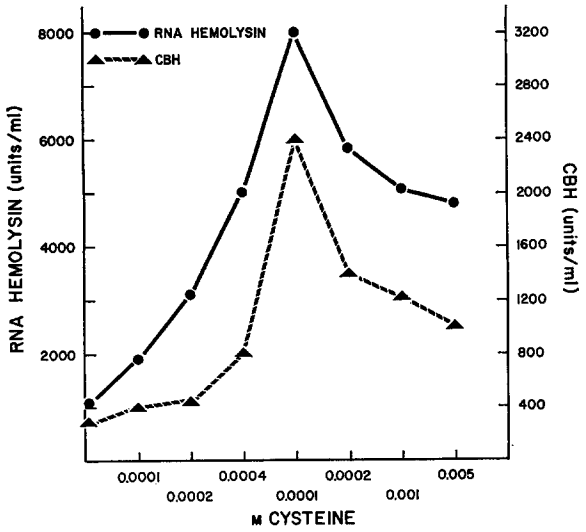

FIG. 1. The effect of the concentration of cysteine on the production of cell-bound hemol ysin and RNA hemolysin.

ponent of the streptococcal cell, but which can also be bound to a variety of carriers which thus become recognized as the oxygen-stable hemolysins (RNA, albumin, tween, and triton hemolysins). Since direct evidence on this point could not be obtained, indirect evidence on such a relationship between the cell-bound hemolysin and streptolysin S was sought by comparing the two kinds of hemolytic agents in terms of conditions of production and conditions of activity, using RNA core hemolysin as a prototype of streptolysin S.

1. Effect on Production of CBH of Various Agents and Conditions Known to Affect the Formation of the RNA Hemolysin.—

(*a*) *Effect of AM components:* As shown elsewhere, maximal amounts of RNA and albumin hemolysins were obtained only in the presence of maltose, Mg^{++}, and cysteine (AM). Table I shows that as in the case of the RNA hemolysin optimal amounts of CBH were obtained only when all components of the activation mixture were present. The only difference in the two systems found was the

relatively higher CBH titers obtained in the presence of Mg^{++} and cysteine alone as compared to the corresponding RNA hemolysin titers. It also appears that low degrees of CBH activity can be observed when no RNA hemolysin can be detected.

(*b*) *Effect of cysteine:* Cysteine and other sulfhydryl compounds have been shown to increase RNA hemolysin formation by resting cells in ratios as high as 10 (7). Fig. 1 shows that cysteine (0.001 M) markedly increased the formation of both CBH and RNA hemolysins. Higher concentrations of cysteine were in-

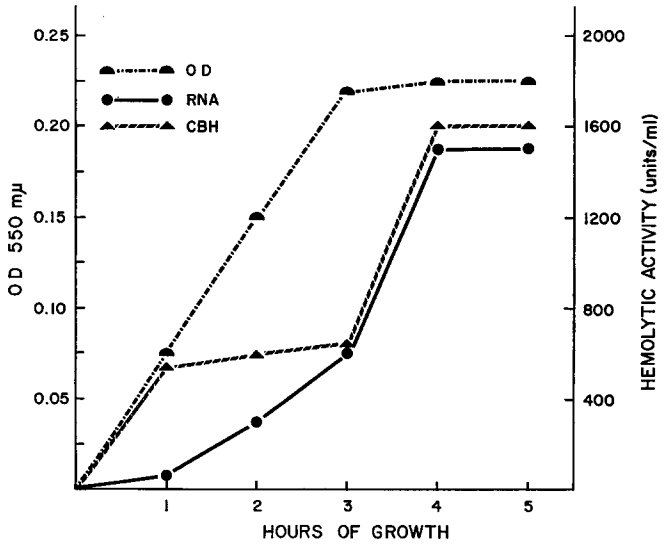

FIG. 2. The production of CBH and RNA hemolysin in relation to the growth phase of streptococci.

hibitory to the two systems. Similar results were obtained in both systems with thioglycolic acid but not by ascorbic acid, indicating that cysteine acts as a donor of sulfhydryl groups (7).

(*c*) *Effect of the age of the culture:* It was shown previously that maximal titers of RNA hemolysin were obtained when the streptococci had reached the stationary phase of growth (8, 5). In order to compare the production of RNA hemolysin and CBH in this regard, streptococci were grown in BHI medium. At 1 hour intervals, 15 ml of culture were withdrawn, the cells were washed with buffer and adjusted to an OD of 0.2 with a spectrophotometer at 550 mμ. Equal portions of such cell suspensions, 0.5 ml, were employed to produce CBH and RNA hemolysin. Fig. 2 shows that maximal CBH and RNA hemolysin titers were both obtained when the streptococci reached the stationary phase of growth. Similar results were obtained with three other streptococcal strains (C203S, Blackmore type 3, and type 6). The titers of both CBH and RNA he-

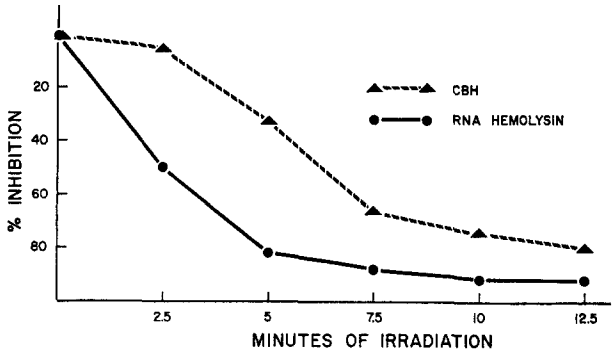

FIG. 3. The effect of ultraviolet irradiation on the production of CBH and of RNA hemolysin.

TABLE II

Effect of Metabolic Inhibitors on the Production of CBH and of RNA Hemolysin

Inhibitor added to streptococci		Hemolytic activity	
		CBH	RNA hemolysin
	μg/ml	*units/ml**	*units/ml**
No inhibitor		2000	2500
Chloramphenicol	100	650	250
"	250	480	80
"	500	300	40
Tetracycline HCl	25	120	0
" "	50	30	0
" "	100	0	0
Merthiolate	50	450	20
"	100	60	5
"	200	50	0

* Inhibitor incubated for 10 minutes at 37°C with streptococci before the addition of AM or of AM and RNA core.

molysin obtained from these strains were much lower than those obtained from strain S84.

(*d*) *Effect of ultraviolet irradiation:* In previous studies (1) it was shown that the formation of the RNA hemolysin was markedly suppressed by UV irradiation, but that irradiation of the preformed hemolysin did not result in loss of hemolytic activity. Accordingly, washed streptococci were irradiated for different periods of time with a mineralight model 41 V ultraviolet lamp from a distance of 12 cm. After various periods of irradiation, portions of the streptococcal suspension were withdrawn and tested for the capacity to produce CBH and RNA hemolysin. Fig. 3 shows that irradiation markedly suppressed the forma-

tion of the two forms of hemolysin. The relative production of CBH was, however, higher after short periods of irradiation.

(*e*) *Effect of metabolic inhibitors:* It was known (1) that various metabolic inhibitors (iodoacetate, tetracyclines, and chloramphenicol) markedly inhibited the formation of the RNA hemolysin but had no effect on its hemolytic activity. Accordingly, the effect of these inhibitors was tested on the production of CBH and RNA hemolysins. It can be seen in Table II that the formation of both hemolysins is affected by tetracyclines, chloramphenicol, and merthiolate. As in the case of the irradiation experiments the formation of CBH is slightly less affected by the inhibitors as compared with RNA hemolysin.

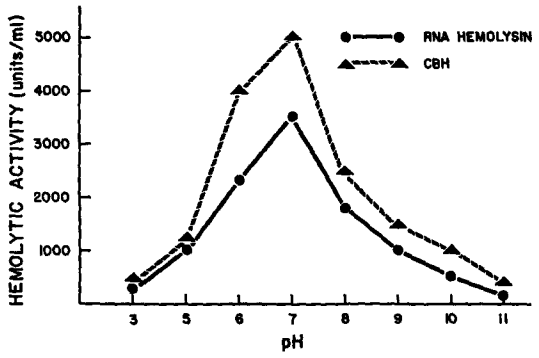

FIG. 4. The production of CBH and RNA hemolysin in relation to pH.

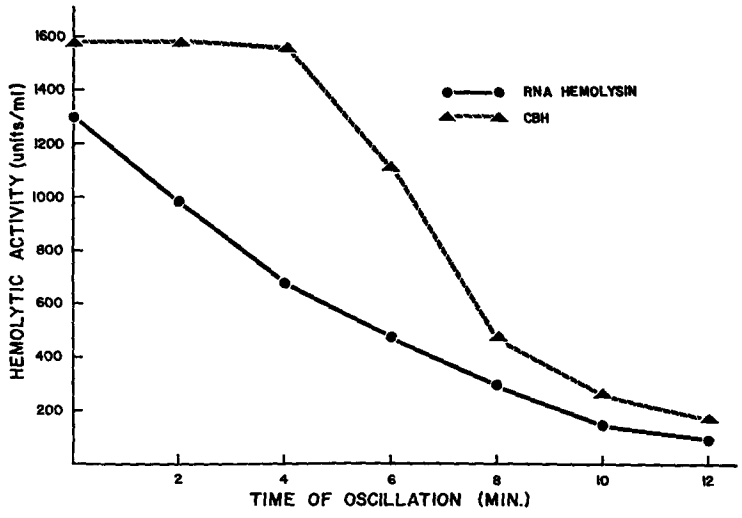

FIG. 5. The effect of sonic oscillation on the production of CBH and RNA hemolysin.

(*f*) *Effect of pH:* Washed streptococci were exposed to a wide range of pH by maintaining aliquots of these for 15 minutes at 37°C in saline buffered at 0.05 M in acetate (pH 3.0 and 5.0), phosphate (pH 6.0, 7.0, and 8.0), or carbonate (pH 9.0, 10.0, and 11.0). Following incubation, to one portion of the cells incubated in the different buffers AM solution was added, while to the others AM and RNA were added. The streptococcal suspensions were further incubated for 30 minutes at 37°C, then the suspension and the RNA hemolysin were respectively

TABLE III

Effect of Certain Inhibitors on the Activity of CBH and of RNA Hemolysin

Streptococci + AM		Hemolytic activity	
		CBH*	RNA hemolysin‡
	μg/ml	*units/ml*	*units/ml*
No inhibitor		2000	5000
Trypan blue	10	100	900
" "	25	20	0
" "	50	0	0
Papain§	25	120	15
"	50	80	15
"	100	50	2
Lecithin‖	1000	260	1000
"	2500	80	550
"	5000	5	300

* Streptococci were serially diluted with buffer. The inhibitors were added to each dilution and incubated for 10 minutes at 37°C before the addition of activation mixture and red blood cells.

‡ Inhibitor incubated for 10 minutes at 37°C with the hemolysin preparation after it was serially diluted.

§ 2 times crystallized, Worthington Biochemical Corp., Freehold, New Jersey.

‖ Vegetable, Nutritional Biochemicals Corp., Cleveland, Ohio.

assayed for hemolytic activity. All the dilutions were performed in saline buffered with phosphate at pH 7.4. As can be seen in Fig. 4, the optimal pH for production of both CBH and RNA hemolysin was 7, and in the case of each hemolysin departures from pH 7 in either direction caused progressive decrease in production.

(*g*) *Effect of sonic disintegration:* Washed streptococci were subjected to sonic disintegration with a 10 kc Raytheon sonic oscillator. Aliquots of cells were removed after different periods of oscillation and used to produce CBH and RNA hemolysin. Fig. 5 shows that oscillation for periods up to 12 minutes affected the formation of both hemolysins. The formation of CBH was, however, more resistant to short periods of oscillation than was that of RNA hemolysin.

2. Effect on CBH of Agents and Conditions Known to Affect the Activity of RNA Hemolysin.—

Effect of antihemolytic inhibitors: Lecithin, trypan blue, and papain have been shown to inhibit RNA hemolysin activity (1, 6). The effect of these inhibitors on CBH was investigated. Preparations of RNA hemolysin having 5000 H. U./ml were incubated with each inhibitor in a range of concentrations. After 10 minutes of incubation at 37°C, the mixtures were titrated for hemolytic activity. In the case of CBH, washed streptococci were serially diluted with buffer, and to each series of dilutions a given amount of inhibitor was added. Following incubation for 10 minutes at 37°C, AM was added to each tube, followed by RBC. Table III shows that all the inhibitors employed markedly inhibited both CBH and RNA hemolysin activity. However, as in the case of the irradiation experiment CBH is less affected than RNA hemolysin.

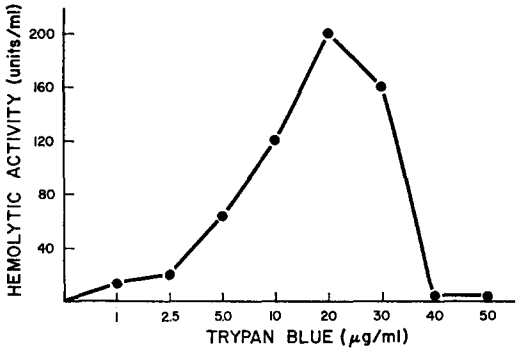

FIG. 6. The induction of hemolytic activity in streptococci by trypan blue.

In the case of the inhbition of RNA hemolysin by trypan blue the following phenomenon was observed: When RNA hemolysin was preincubated with 25 μg/ml of the dye and then diluted serially to determine the extent of inhibition, it was found that up to the dilution of 160 no trace of hemolysis occurred. Partial hemolysis was found at dilutions of 320 and 640, and at higher dilutions there was, again, no hemolysis. Similarly, the same amount of hemolysin preincubated with 10 μg/ml of trypan blue showed hemolytic activity in dilutions of the mixture between 8 and 128, but not at lower or higher dilutions. In previous studies it was shown that various materials can serve as carriers for the hemolytic moiety synthesized by streptotocci and that it was possible to transfer the hemolytic activity from one carrier to another within the group. The possibility was therefore examined that trypan blue, although an inhibitor of RNA hemolysin, can act at low concentrations as a carrier of the hemolytic moiety. Washed streptococci were incubated with AM and with various concentrations of trypan blue (1 to 50 μg/ml). After 20 minutes of incubation at 37°C the cells were removed by high-speed centrifugation and the supernate was passed through a Millipore filter and assayed for hemolytic activity. Fig. 6 shows that trypan blue at 1 to 20 μg/ml can induce hemolytic activity from streptococci in a manner similar to that observed for albumin, tween, or RNA. Since the hemolysin induced by trypan blue was completely inhibited by excess trypan blue (>30 μg/ml) and by papain and lecithin, this dye was recognized as an additional carrier in the group of oxygen-stable streptococcal hemolysins. Under similar conditions lecithin failed to induce hemolytic activity from washed streptococci incubated with AM.

3. Susceptibility of Red Blood Cells of Various Animal Species.—Differences in susceptibility of RBC obtained from different animal species to bacterial and snake venom hemolysins have been described (6, 9). To further study the relationship of the CBH to RNA hemolysin the susceptibility of RBC from different animals to CBH and RNA hemolysin was tested. Blood was obtained in heparin from the various animals and used on the same day. A 2 per cent suspension of the various RBC was prepared in saline buffered with 0.05 M phosphate pH 7.4. The cell suspension was then adjusted to contain the same amount of hemoglobin. The hemolytic effect, in units per ml, was determined for the erythrocytes of each of the mammalian species tested, and these are shown as ratios to the hemolytic effect on human erythrocytes, in the upper part of Table IV. It can be seen that in each case the ratio is greater than 1 and that the relative susceptibility of the respective mammalian cells to CBH and RNA hemolysin does not exceed a factor of 2. On the other hand, in the case of the two species of nucleated RBC (chicken and frog), the susceptibility to RNA hemolysin was much lower (6- to 20-fold) than to CBH. The experiments were repeated with the number of frog and chicken red cells varied over an 8-fold range with similar results. A similar observation of relatively lower susceptibility to RNA hemolysin than to CBH was also found in the case of Ehrlich ascites tumor cells. These experiments indicate that nucleated RBC behave differently from the mammalian RBC in their relative susceptibility to CBH and RNA hemolysin.

TABLE IV

Relative Susceptibility of RBC of Various Animal Species to CBH and RNA Hemolysin

Source of red cells	Ratio of hemolytic units for RBC of various species to that for human RBC	
	CBH	RNA hemolysin
Human	1.00	1.00
Rabbit	1.30	1.20
Chicken	1.66	0.25
Goat	1.80	1.20
Rat	2.20	2.90
Mouse	4.00	3.10
Cat	2.40	1.20
Sheep	1.00	1.10
Chicken	1.60	0.25
Frog*	0.30	0.015

* Similar results were obtained when frog-ringer solution was used instead of phosphate saline buffer.

4. Absence of CBH in Strain C203U.—Bernheimer (6) has shown that strain

C203U obtained by mutation from the parent strain C203S type 3 fails to produce RNA hemolysin. Also it was previously shown that this mutant fails to produce either the albumin, tween, or triton hemolysins (1). In the present study it was also found that strain C203U also fails to show any trace of CBH activity even if used in very thick suspensions. On the other hand, strain Blackmore type II, known to produce streptolysin S, does not produce streptolysin O, and has high CBH activity (6). In other studies (unpublished) it was found that 100 Group A strains tested produce CBH and RNA hemolysin and no streptococcal strain was found to produce CBH without producing RNA hemolysin. It is, however, important to stress that the amounts of CBH and RNA hemolysin produced by different strains vary markedly.

DISCUSSION

The experiments described in this paper were performed in order to obtain evidence on the question of whether the cell-bound hemolysin (CBH) represents that hemolytic moiety synthesized by streptococci which becomes recognized as streptolysin S (RNA, albumin, tween, and triton hemolysins) when any of a series of carrier molecules are added to washed cells. Evidence for the similarity of the two forms of hemolysin was obtained in experiments on the formation of CBH and RNA hemolysin and on the activity on the two hemolytic systems.

The formation of the two hemolytic agents was found to be inhibited to a large extent by metabolic inhibitors (Table II), by ultraviolet irradiation (Fig. 3), and by sonic oscillation (Fig. 5). Also, the formation of both hemolysins was pH-dependent (Fig. 4), and was of maximal degree only if optimal amounts of maltose, Mg^{++}, and sulfhydryl compounds are present (Table I, Fig. 1). Finally, it was shown that maximal formation of the two forms of hemolysins takes place during the stationary phase of growth (Fig. 2) and that both hemolysins are absent from a streptococcal mutant C203U. However, since all these data are derived from experiments employing nonspecific metabolic inhibitors capable of affecting different metabolic pathways, the possibility cannot be excluded that although similar in many respects, the two forms of hemolysin may represent two different hemolysins produced by very similar metabolic processes.

A source of difficulty in the comparison of the two forms of hemolysins stems from the fact that while the RNA and other forms of the oxygen-stable hemolysins are estimated in solution, CBH must be estimated in the presence of the streptococci, under conditions in which it is not possible at present to eliminate entirely the effect of continued synthesis of the CBH at a low rate during the test procedure itself. In this connection it is of interest that in experiments in which resting streptococci, unaltered by the experimental procedure, were producing CBH and RNA hemolysin under varying environmental conditions, such as pH, or concentration of cysteine, quite uniform pairs of curves were found (Figs. 1 and 4), whereas in experiments in which the state of the streptococci themselves was being altered, as by UV irradiation or

sonic oscillation, relatively more CBH than RNA hemolysin was found in some parts of the curves, those involving conditions of moderate degrees of injury to the streptococcal cells (Figs. 3 and 5). Another problem raised by the presence of streptococcal cells in the reaction mixtures which are used to measure CBH, is that it is not possible, under these circumstances to be certain that lysis by CBH does not involve additional cell-bound or cell-free factors. (Experiments on this point are included in the following paper.)

In comparisons of hemolytic activity of the two systems, on the other hand, this source of difficulty is not encountered. The fact that trypan blue and lecithin block the hemolytic activity of both CBH and the RNA hemolysin (these agents do not affect the synthesis of the RNA hemolysin), and the findings that crystalline papain inactivates both hemolysins, favor the interpretation that all these agents affect the same hemolytic moiety, probably a peptide in nature (4). The suggestion of the peptide nature of the active group is consistent with the findings of Koyama and Egami (10, 11) that the RNA hemolysin is a complex of an oligonucleotide and a peptide, and with the findings of Ginsburg and Harris (4) that papain inactivates albumin, tween, or triton hemolysins as well as the hemolytic moiety transferred from RNA to either tween or triton.

The finding that red blood cells obtained from various mammals are similarly susceptible to both CBH and to RNA hemolysin also points to the similarity of these two forms of hemolysins. On the other hand, the data that red blood cells of chickens and frogs are more susceptible to CBH than to RNA hemolysin raises further difficulties in any generalization. Since similar results were also obtained with Ehrlich ascites tumor cells it is possible that this phenomenon is characteristic for nucleated cells.

A final conclusion on the identity of the CBH with the hemolytic moiety attached to the various carriers will probably be reached only when the active material in CBH can be isolated and its nature established.

It was of interest that trypan blue, although a potent inhibitor of streptolysin S, can at low concentrations serve as a carrier of hemolysin; i.e., an inducer of hemolytic activity from washed streptococci. This observation also indicates that yet other molecular species may be found to have the property of inducing oxygen-stable streptococcal hemolysin, and also gives further support to the hypothesis that the oxygen-stable streptococcal hemolysins are complexes of a non-specific carrier with a specific hemolytic moiety synthesized by streptococci.

SUMMARY

The relationship of the streptococcal hemolysin which is recognized on incubation of RBC with streptococcal cells (cell-bound hemolysin, CBH), to RNA hemolysin, a representative of oxygen-stable hemolysin (streptolysin S) has been studied. A number of similarities have been found in the conditions for optimal production of each of these hemolysins, a requirement for cysteine, Mg^{++}, and glucose; maximal production by streptococci in the stationary phase; similar curves of pH-dependence. In both systems, the production of hemolysin

was inhibited by certain antibiotics, by ultraviolet irradiation, and by sonic disruption and was absent in the same streptococcal mutant strain. The hemolytic activity of both systems was inhibited by lecithin, trypan blue, and papain. Similarities were also found in relative susceptibilities to the two hemolytic systems of erythrocytes of a number of animal species.

These data support a suggestion advanced in an earlier study that a streptococcal hemolytic moiety, which can be induced by, and carried on, a number of diverse agents to comprise the group of oxygen-stable hemolysins, serves, in its original attachment to a component of the streptococcal cell, to produce the hemolytic effect recognized as the cell-bound hemolysin.

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