STUDIES ON THE FLEXNER GROUP OF DYSENTERY BACILLI

VI. The Detoxification of Shigella paradysenteriae by Means of Periodic Acid

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Plate 31

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The problems encountered in immunizing human beings to the Shiga bacillus are different from those associated with vaccination against the Flexner group of dysentery bacilli. The Shiga bacillus elaborates both an endo- and an exotoxin (1), and the latter can be readily detoxified by treatment with formalin (2). There is reason to believe that this toxoid might be effective in controlling the disease in man (3). Flexner bacilli, on the other hand, elaborate only on endotoxin (4) which cannot be attenuated by the usual procedures; as a result, no thoroughly satisfactory method had been devised prior to the second World War which would render microorganisms of this group innocuous. Although extensive experimentation has been carried out in which untreated Flexner bacilli have been employed for vaccination, the effectiveness of the procedure is still unknown. This is due in part to the toxic nature of the vaccines themselves which renders it difficult to administer quantities sufficiently great to elicit a maximum antibody response. In addition, the extensive number of specific bacterial types (5) encountered in epidemic areas likewise make the selection of appropriate microorganisms difficult. If it were possible, however, to select wisely representative bacterial types, and at the same time to reduce their toxicity without impairing too much their antigenic efficacy, the problem of immunizing peoples effectively would be considerably facilitated, and the subsequent evaluation of such procedures should be less difficult.

Numerous investigators have attempted to devise methods for detoxifying dysentery bacilli and some have not been without success. Thus, in 1916 Dean and Adamson (6) found that treatment with sodium hypochlorite or with hydrogen peroxide caused a diminution in the toxicity of dysentery bacilli without destroying their ability to incite antibodies. More recently a preliminary account of the detoxification of the somatic antigen of *Shigella paradysenteriae* Type Z using hydrogen peroxide and ultraviolet irradiation, has appeared (7). Mention should also be made of the work of Tamura and Boyd (8) who treated Shiga bacilli with ketene gas and found that their toxic properties were lost, and of the exacting work of Treffers (9) who has succeeded in detoxifying the somatic antigen of this microorganism, and that of *E. typhosa* as well, by acetylation with acetic anhydride and pyridine. In

addition, the detoxification of dysentery organisms by ultraviolet irradiation (10), and the ineffectual use of such vaccines for the immunization of human beings to experimentally induced dysentery infections, have recently been reported (11).

In the present communication we are concerned with the description of a method for the detoxification of dysentery bacilli of the Flexner group. Two representative microorganisms have been chosen for study, Type Z and sp. Newcastle. When bacilli of either of these types are treated with 0.01 M periodic acid at pH 5.0, their toxic properties, as determined both by intraperitoneal injection of mice, and by skin test in rabbits, are rapidly destroyed, yet their capacity to incite specific immune bodies in experimental animals is not.

EXPERIMENTAL

Materials and Methods.—Strains of the two specific types of *Sh. paradysenteriae* Type Z and sp. Newcastle used in this study were procured from the U. S. Army Medical Center. The bacilli were smooth variants and were cultivated on neopeptone agar for 18 hours at 37°C. The microorganisms were washed from the surface of the medium, separated by centrifugation, suspended in distilled water, killed by heating at 60° for 30 minutes, and finally frozen and dried. Toxicity tests of treated and untreated bacilli were performed by injecting a suspension of weighed amounts of microorganisms intraperitoneally into 20 to 22 gm. white mice (Rockefeller Institute strain). All animals were observed for 5 days, and deaths and survivals recorded. Antisera to the treated and untreated bacilli were prepared by injecting a suspension of the microorganisms into rabbits intravenously on alternate days. Each animal received doses of 10, 40, and 100 micrograms. Seven days after the last injection the animals were bled and the sera collected. Precipitin and agglutination tests were performed in the conventional manner. The quantitative assay of the antisera was made by a photometric technique using a phototurbidimeter as previously described (4, 12). The quantitative determination of periodic acid was made by adding measured aliquots of the solution to be determined to an excess of 0.02 N standard sodium arsenite solution in the presence of an excess of sodium bicarbonate and potassium iodide. The unconsumed arsenite was titrated with 0.02 N iodine-potassium iodide solution (13).

The Action of Periodic Acid on Sh. paradysenteriae Type Z and Sp. Newcastle.—During our investigations on the chemical degradation of the somatic antigen of *Sh. paradysenteriae* Type Z, it was observed that when the antigen was treated for a period of 120 minutes with 0.1 M periodic acid at pH 5.0 (0.1 M acetate buffer) the serological activity was rapidly destroyed and at the same time a marked diminution in toxicity occurred. The resulting reaction product was soluble, but on dialysis, followed by freezing and drying, the product became for the most part extremely insoluble, even at alkaline pH. That a marked chemical change had taken place was evident, for not only were much of the immunological and toxic properties of the antigen changed, but the end-product gave a brilliant color when treated with Schiff's reagent. This material had suffered such drastic changes that it no longer served well as an immunizing

agent. It was decided, therefore, to investigate the action of periodic acid on the microorganisms themselves.

Agglutination.—A suspension of 100 mg. of Type Z bacilli in 90 ml. of 0.1 M acetate buffer at pH 5.0 was treated with 10 ml. of 0.1 M periodic acid. At intervals of 10, 40, and 160 minutes samples were removed, and an excess of aqueous glycerol was added to destroy the periodate. The bacilli were centrifuged, washed twice, and finally resuspended in 0.9 per cent NaCl. Their agglutination reactions in a standard Type Z serum were studied and compared with those of untreated cells.

An identical experiment was performed with sp. Newcastle organisms. The results of both experiments are recorded in Table I where it is seen that contact

TABLE I

Agglutination of Sh. paradysenteriae Type Z and Sp. Newcastle in Homologous Antiserum before and after Treatment with Periodic Acid

Bacteria in contact with HIO_4 for	Microorganism tested	Final dilution of antiserum					
		1:200	1:400	1:800	1:1600	1:3200	1:6400
min.							
0 (control)	Type Z	++++	++++	+++	+±	±	0
10		++++	+++	++	±	0	0
40		+++	++	±	0	0	0
160		+	+	0	0	0	0
0 (control)	Sp. Newcastle	++++	++++	+++	++	+	0
10		++±	++	+	±	0	0
40		+±	+±	±	0	0	0
160		+	+	0	0	0	0

++++ = complete agglutination, clear supernate.
0 = no agglutination.

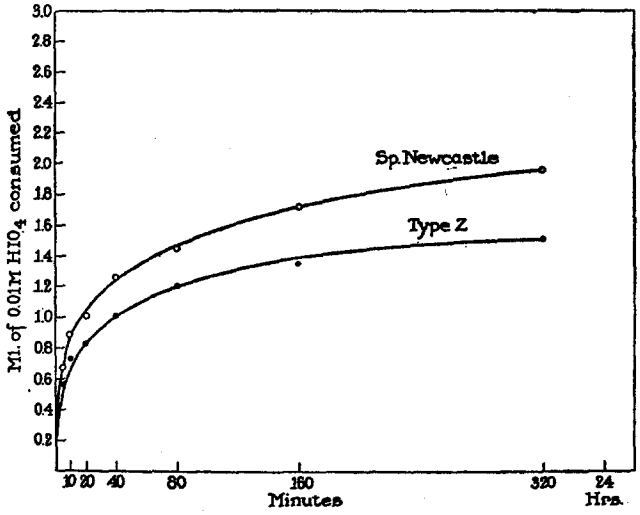
with periodic acid causes a marked diminution in the agglutinability of the cells in homologous antiserum.

Consumption of Periodic Acid by Sh. paradysenteriae Type Z and Sp. Newcastle.—In order to determine the rate at which periodic acid is consumed by the Type Z and sp. Newcastle bacilli a suspension of 0.2 gm. of dried bacteria in 90 ml. of 0.1 M acetate buffer at pH 5.0 was made. 10.0 ml. of 0.1 M periodic acid were added and the mixture was stirred mechanically. 10 ml. aliquots were removed at varying time intervals and added to 10.0 ml. of 0.02 N sodium arsenite, 10 ml. of 1 N sodium bicarbonate, and 1 ml. of 20 per cent potassium iodide. After standing 15 minutes the excess arsenite was titrated with standard 0.02 N iodine-potassium iodide solution. The amount in milliliters of 0.01 M periodic acid consumed by the 20 mg. sample of bacilli was calculated, and the results recorded in Text-fig. 1.

It can be seen from the figure that the consumption of periodic acid is rapid indeed and that the loss in agglutinability (Table I) follows roughly the

consumption of the reagent. The reaction between bacilli and reagent appears to be complete within 24 hours, for measurements made thereafter show only negligible increases.

Destruction of the Toxic Component.—0.5 gm. of killed bacilli of both types was treated with 0.01 M periodic acid under conditions identical with those described above. After 10, 40, and 160 minutes a third of the reaction mixture was removed and an excess of aqueous glycerol added. A control experiment; *i.e.*, bacilli suspended in buffer solution, was likewise performed, without the addition of periodic acid. The bacilli were separated, washed thoroughly, then frozen and dried. The toxicity of the control and treated microorganisms was tested in mice and the results recorded in Table II.



TEXT-FIG. 1. Milliliters of 0.01 M HIO_4 consumed by 20 mg. of *Sh. paradysenteriae* Type Z and sp. Newcastle.

From the results presented in Table II it is evident that the toxicity of the cells as determined by intraperitoneal injection of mice, diminishes rapidly on contact with periodic acid.

Immunization of Rabbits with Sh. paradysenteriae Type Z before and after Treatment with Periodic Acid.—A group of rabbits was immunized by the intravenous injection of *Sh. paradysenteriae* Type Z before and after treatment with 0.01 M periodic acid under the conditions described above. Each group contained three animals, and was injected with untreated bacilli (control group), and with bacilli treated for 10, 40, and 160 minutes respectively. The animals received 10, 40, and 100 micrograms of microorganisms intravenously and were bled 7 days following the last injection. The sera were then tested for bacterial agglutinins with homologous vaccine, and the results recorded in Table III.

It can be seen that the animals immunized with treated bacilli evoke antibodies which agglutinate the homologous treated bacterial cells. It is evident,

however, that the agglutination reactions of the sera evoked by bacilli treated with periodic acid are by no means as strong as those of the control group. Furthermore, the ability of treated cells to evoke homologous agglutinins diminishes the longer the bacilli remain in contact with the reagent. If one

TABLE II

Toxicity of Sh. paradysenteriae Type Z and of Sp. Newcastle before and after Treatment with 0.01 M Periodic Acid

Bacteria in contact with HIO_4 for	Microorganism tested	Bacteria injected, *mg.*				
		5.0	2.5	1.25	0.62	0.31
min.						
0 (control)	Type Z	–	D_{18} D_{18} D_{18} D_{18}	D_{18} D_{18} D_{18} D_{18}	D_{18} D_{6} S S	S S S S
10		D_{18} D_{18} D_{40} S	D_{18} D_{18} S S	S S S S	S S S S	–
40		D_{18} S S S	S S S S	S S S S	S S S S	–
160		S S S S	S S S S	S S S S	S S S S	–
		8.0	4.0	2.0	1.0	0.5
0 (control)	Sp. Newcastle	–	–	D_{18} D_{18} D_{18} D_{40}	D_{18} D_{18} D_{24} S	S S S S
10		D_{18} D_{40} S S	D_{24} S S S	S S S S	S S S S	–
40		S S S S	S S S S	S S S S	S S S D_{40}	–
160		S S S S	S S S S	S S S S	S S S S	–

D = death. Subscript denotes hours elapsed before death occurred.
S = survival.

TABLE III

Agglutination of Treated Bacilli in Sera of Rabbits Immunized with Sh. paradysenteriae Type Z Oxidized with 0.01 M Periodic Acid

Serum No.	Rabbits injected with bacteria in contact with HIO_4 for	Final dilution of antiserum					
		1:200	1:400	1:800	1:1600	1:3200	1:6400
	min.						
44	10	++±	++±	++±	++	±	0
47	40	++	++	++	++±	+	0
49	160	+	++	++	++	+	0

In each instance the test antigen used was the same as that used for injection.

now tests these sera with untreated Type Z bacilli (Table IV) the latter are readily agglutinated, and more strongly than are the homologous treated cells.

When these same sera are tested for their ability to precipitate the native Type Z somatic antigen, they readily precipitate the latter (Table V), and the capacity of precipitation follows the same course as does the agglutination reaction. For purposes of comparison a group of rabbits was also immunized with the sp. Newcastle bacilli, before and after treatment for 40 minutes with periodic acid. The treated bacilli gave rise to antisera as potent in agglutinins

and precipitins as those evoked by untreated bacilli. The protocols need not be given.

From the results of these experiments it can be concluded that the *Sh. paradysenteriae* Type Z, when treated with periodic acid, still retains its ability to incite antibodies which agglutinate both treated and untreated bacilli. The antibodies evoked are those directed toward the somatic antigen, for it is evident that the latter precipitates readily in all the antisera tested. These same

TABLE IV

Agglutination Reactions of Sera of Rabbits Immunized with Sh. paradysenteriae Type Z Treated with 0.01 M Periodic Acid, Using Untreated Type Z Bacilli as the Test Antigen

Serum No.	Rabbits immunized with bacteria in contact with HIO_4 for	Final dilution of antiserum					
		1:200	1:400	1:800	1:1600	1:3200	1:6400
	min.						
44	10	++++	+++	++±	++	±	0
47	40	++++	++	++	++	±	0
49	160	+++	++	++	+±	0	0
51	0 (control)	++++	++++	++++	++	±	0

TABLE V

Precipitin Reactions of Sera of Rabbits Immunized with Sh. paradysenteriae Type Z Treated with 0.01 M Periodic Acid

Serum No.	Rabbits immunized with bacteria in contact with HIO_4 for	Final dilution of test antigen				
		1:2000	1:10,000	1:50,000	1:250,000	1:1,000,000
	min.					
44	10	++++	++++	+++	++	±
47	40	++++	++++	+++	+±	±
49	160	+++	+++	++	+	0
51	0 (control)	++++	++++	+++	++	±

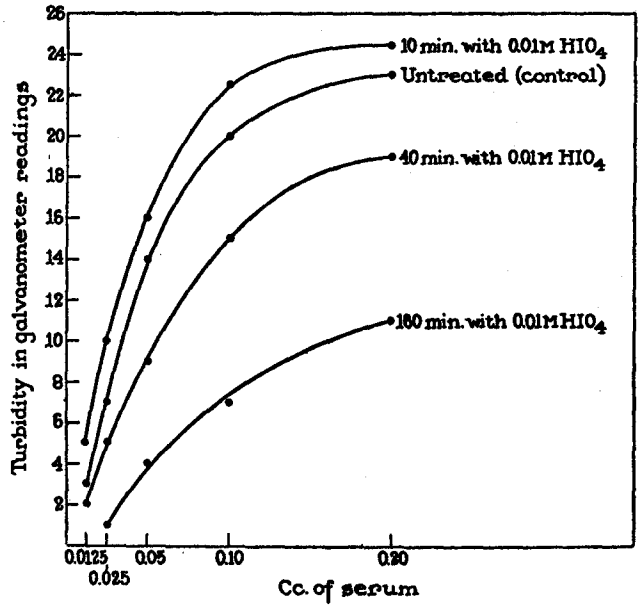
experiments have been repeated using the Newcastle organism in place of Type Z. The results were identical with those just described.

Quantitative Estimation of Antibodies Evoked by Type Z Bacilli after Treatment with Periodic Acid.—It is impossible to assay the antibody content of the immune sera as described solely by qualitative measurements. It was advisable, therefore, to determine quantitatively the antibody content of the sera obtained from the various groups of animals injected with treated bacilli and compare it with that of the sera of rabbits immunized with untreated organisms.

The sera from each group of rabbits were pooled, and the antibody content was determined photometrically (12), using the chemically purified Type Z antigen as precipitinigen. The results are presented graphically in Text-fig. 2 where it can be seen that animals immunized

with minimal, and identical quantities of bacilli exposed to periodic acid for increasing time intervals, give rise to decreasing amounts of antibodies. It was pointed out in Table II that bacilli exposed for 10 minutes to 0.01 M periodic acid, suffered a decrease in toxicity of approximately 75 per cent. Yet these same microorganisms gave rise to sera as potent as those elicited by untreated bacilli. Bacteria exposed for 40 minutes had only a tenth of the toxicity of the untreated bacilli, yet they gave rise to antisera with half the potency of those elicited by the fully toxic untreated microorganisms.

From this experiment it can be concluded that exposure of Type Z dysentery bacilli for short intervals to 0.01 M periodic acid markedly decreases their



TEXT-FIG. 2. Turbidimetric antibody titre of sera of rabbits immunized with *Sh. paradysenteriae* before and after treatment with 0.01 M HIO_4.

toxicity without impairing their ability too greatly to evoke type-specific immune bodies.

Extraction of a Soluble Antigen from Sh. paradysenteriae Type Z after Treatment with Periodic Acid.—In order to determine whether Type Z bacilli previously treated with 0.01 M periodic acid would yield a soluble antigen capable of eliciting specific immune bodies in experimental animals, the following experiment was performed.

10 gm. of Type Z bacilli grown in a peptone-blood extract (14) medium and killed with 1 per cent formalin, were suspended in 300 ml. of water. 200 ml. of 0.5 M acetate buffer at pH 5.0, and 100 ml. of 0.1 M periodic acid were added. The final pH of the solution was 5.0. The mixture stood for 40 minutes then 5 ml. of glycerol were added. The bacilli were removed

by centrifugation, washed, and extracted with 500 ml. of 50 per cent pyridine at 37° for 24 hours. The pyridine extract was separated from the bacterial bodies by centrifugation. The solution was then thoroughly dialyzed, concentrated *in vacuo*, and the material was finally frozen and dried. 1.9 gm. of substance were recovered. This material was analyzed for nitrogen (2.8 per cent) and phosphorus (0.6 per cent) and tested for toxicity in mice. It required 10 mg. of the extracted product to kill mice. None of the animals which had received 5.0 mg. or less died.

The extracted substance was pale yellow in color and appeared to be a mixture of oxidation products of the somatic antigen and its hapten. Aqueous solutions could be readily fractionated with acetone. About half the total material was insoluble in 50 per cent acetone (fraction I, hereafter called Fr. I). This substance contained 5.8 per cent nitrogen and 1.8 per cent phosphorus and approximated in its analysis the unaltered somatic antigen of the Type Z bacillus. Lesser quantities of material precipitated at 66 (Fr. II) and 75 (Fr. III) per cent acetone concentrations. The nitrogen content of these fractions was 2.8 and 1.05 per cent and the phosphorus content 0.6 and 0.1 per cent respectively. All of the fractions, in dilutions as high as 1:50,000, precipitated in an antiserum to the unaltered Type Z bacillus, but the precipitation was by no means as heavy as that obtained with comparable dilutions of purified untreated somatic antigen. All of the fractions gave a color when tested with Schiff's reagent, the intensity varied inversely with the nitrogen content of the fraction tested. The biuret reaction was intense only with Fr. I. Fr. II gave a feeble test whereas Fr. III gave none. When tested for toxicity, none of the fractions killed mice in quantities of 10 mg. or less save Fr. I, where one of four mice died. In this respect it is well to record that the unaltered somatic antigen of the Type Z bacillus kills mice in quantities of 250 micrograms.

It is postulated that the treatment of Type Z bacilli with periodic acid brings about a rapid oxidation of the carbohydrate moiety of the somatic antigen. At the same time the latter may suffer dissociation to some extent. When the treated bacilli are extracted with aqueous pyridine some intact but oxidized lipocarbohydrate-protein complex is obtained (Fr. I). Fr. II and III probably represent products of degradation in which the carbohydrate has likewise been oxidized to a polyaldehyde. Although Fr. I was repeatedly precipitated with one volume of acetone, yet its toxic, immunologic, and analytical properties remained unaltered.

Toxicity of the Antigenic Fraction as Determined by Intradermal Tests in Rabbits.—As shown above the antigenic fraction obtained from Type Z bacilli treated with periodic acid (Fr. I) has a low order of toxicity when injected intraperitoneally into mice. This same material when administered intravenously to rabbits failed to call forth toxic symptoms in quantities of 500 micrograms. Another method of assaying the toxicity of the somatic antigens of the *Sh. paradysenteriae* is the reaction caused by the intradermal injection of the material in rabbits. Although the lethal dose of the Type Z antigen for rabbits, when administered intravenously, is approximately 25 micrograms, this animal withstands far greater quantities intradermally. By this route quantities of 1000 micrograms are tolerated without causing death, but in most instances the animals suffer a generalized reaction with indolence, elevation in temperature, etc.

In order to test the severity of the local reaction caused by the unaltered Type Z somatic antigen and compare it with that induced by the fraction obtained from treated bacilli (Fr. I), several rabbits received on the right side of the body 1000, 500, and 250 micrograms of the native antigen; on the opposite side the same quantities of the extraction product (Fr. I) obtained from periodate-treated cells were injected. The stated dose was dissolved in 0.2 ml. of sterile saline, and the injections were made 5 cm. apart. At the sites injected with untreated antigen there appeared within 48 hours, large raised, boggy red areas a centimeter or more in diameter which showed central necrosis. The reaction was characterized by the presence of much edema and circumferential erythema. The intensity of the reaction was proportional to the quantity of material injected, and was most severe at the site injected with 1000 micrograms as can be seen in Fig. 1*a* (extreme right). The injection of the antigenic material obtained from bacilli treated with periodic acid produced only small circumscribed, reddened papules which were firm (Fig. 1*b*). The reactions in these instances were characterized by the absence of edema and necrosis. The photographs do not adequately show the differences in the lesions.

TABLE VI

Agglutination of Sh. paradysenteriae Type Z in Sera of Rabbits Immunized with Extraction Product

Serum No.	Final dilution of antiserum					
	1:200	1:400	1:800	1:1600	1:3200	1:6400
73	++++	++++	++++	+++	+	0
74	++++	++++	++++	++++	++	±
75	++++	++++	++++	+++	+	0

Immunization of Rabbits with the Product of Oxidation of the Type Z Somatic Antigen.—Because Fr. I as described above showed a definite serological activity it was decided to immunize animals with the material.

A group of rabbits was given a total of 4.0 mg., administered on 5 alternate days, the first two doses given were 0.5 mg. each. It should be noted that this quantity of material is massive in terms of unaltered antigen, from the point of view not only of the amount necessary to elicit antibodies, but also of its toxicity since it represents approximately 20 lethal doses for the rabbit. Yet the animals injected with the oxidized material showed no severe reactions. Seven days after the last injection the sera of the rabbits were tested both for precipitins and bacterial agglutinins. The results are recorded in Tables VI and VII.

The tabulated tests reveal that the product of oxidation of the Type Z somatic antigen obtained from bacilli treated with periodic acid is antigenic in rabbits; it evokes antibodies which precipitate the unaltered antigen, and agglutinate untreated Type Z bacilli. In addition, these same antisera precipitate both the native somatic antigen of the Type Z bacillus, and the product of oxidation obtained from treated cells.

If the most potent of the three sera described above (serum 74) is compared with a typical immune serum prepared by prolonged immunization of rabbits

with untreated Type Z bacilli, its antibody content, as determined photometrically, is practically identical with that of the latter. It can be concluded, therefore, that the relatively non-toxic oxidized somatic antigen of *Sh. paradysenteriae* Type Z is an efficacious agent for the production of potent dysentery antisera.

TABLE VII

Precipitin Reactions of Sera of Rabbits Immunized with Extraction Product of Treated Type Z Bacilli

Serum No.	Test antigen used	Final dilution of test antigen				
		1:2000	1:10,000	1:50,000	1:250,000	1:1,000,000
73	Unaltered Type Z somatic antigen	+++	+++	++±	+	0
74		++++	+++	+++	++	+
75		+++	+++	++	+	0
73	Oxidized Type Z somatic antigen	+++	++	+	0	0
74		++++	+++	++	±	0
75		+++	++	+	0	0

TABLE VIII

Agglutination Reactions of an Antiserum to Treated Bacilli before and after Absorption

Serum absorbed with	Test antigen used	Final dilution of antiserum								
		1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560
Unabsorbed	Type Z (HIO_4) bacilli	−	−	−	−	++++	++++	+++	+	0
	Type Z bacilli	−	−	−	−	++++	++++	++++	+++	+
Type Z (HIO_4) bacilli	Type Z (HIO_4) bacilli	++	++	+	0	0	0	0	−	−
	Type Z bacilli	++++	++++	++++	++++	+++	++	0	−	−
Untreated Type Z bacilli	Type Z (HIO_4) bacilli	++++	++++	++++	+++	++	+±	0	−	−
	Type Z bacilli	0	0	0	0	0	0	0	−	−

It was shown above that the antigen obtained from Type Z bacilli previously treated with periodic acid elicits antibodies which agglutinate both untreated and treated bacilli. It has likewise been demonstrated that bacilli treated with periodic acid lose their ability to agglutinate in Type Z antiserum. It would appear therefore that the antibodies produced by the treated bacilli are of a

special type and differ from those present in true Type Z immune serum. The results of cross-absorption tests to test this assumption are given.

The pooled sera of rabbits injected with Type Z bacilli previously treated for 40 minutes with 0.01 M periodic acid were absorbed with (*a*) Type Z bacilli, and (*b*) Type Z bacilli treated for 40 minutes with 0.01 M periodic acid. The serum was now tested for its ability to agglutinate both treated and untreated bacilli.

The results presented in Table VIII reveal that the serum of rabbits immunized with treated bacilli, when absorbed with the latter, still agglutinates untreated bacilli. Although the titre has been considerably reduced (from 1:2560 to 1:640), it is yet not possible to remove all the antibodies by absorption with the homologous antigen. Likewise, when the immune serum is first absorbed with the heterologous untreated Type Z bacilli, there are left in solution antibodies which agglutinate the homologous treated bacilli, though again the titre is considerably reduced, from 1:1280 to 1:320. From these experiments one may deduce that Type Z bacilli treated with 0.01 M periodic acid for a period of 40 minutes give rise in rabbits to antibodies with different specificities. Some of these antibodies are directed against the chemically altered antigen, whereas others appear to be directed against the native unaltered somatic antigen. The explanation for this phenomenon will be discussed later.

DISCUSSION

Periodic acid has long been used in the study of the structure of organic derivatives having special molecular groupings. Compounds having two hydroxyl groups or an hydroxyl and amino group on adjacent carbon atoms are readily oxidized to the corresponding aldehydes according to the following equations:

$$RCHOH—CHOHR' + HIO_4 \rightarrow RCHO + R'CHO + H_2O + HIO_3$$

$$RCHOH—CHNH_2R' + HIO_4 \rightarrow RCHO + R'CHO + NH_3 + HIO_3$$

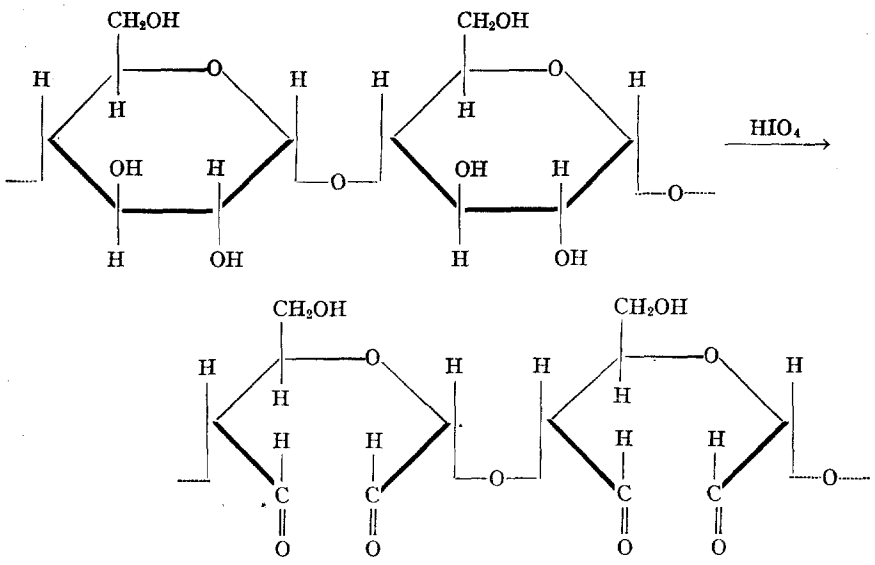
It will be noted in both instances that the carbon-carbon bond has suffered a cleavage (15).

In the field of carbohydrate chemistry in particular, periodic acid has been extensively employed in the study of the structure of the derivatives of mono- and disaccharides. The mode of action of this remarkable reagent on the more complex, naturally occurring substances has, however, not been thoroughly explored, yet much valuable though less exacting information has been gained through employing this procedure, particularly in the study of polysaccharides.

In order to appreciate more fully the profound chemical changes which take

place whan a complex polysaccharide is treated with periodic acid, it is well to review the changes which ensue when starch is oxidized with the reagent (16).

When a suspension of starch is treated with aqueous periodic acid it consumes within an hour approximately 0.8 of a mole of the reagent, and within 24 hours one mole. The treated starch granules themselves fail to show the characteristic cross when observed with transmitted polarized light, and the material has lost its ability to color blue with iodine, yet the oxidized product retains its colloidal properties. The starch unit itself has been oxidized to a dialdehyde and the intramolecular gluosidic linkages have presumably not been severed:



During our studies on the somatic antigens of *Sh. paradysenteriae* we were in search of a method for dissociating the lipocarbohydrate-protein complex more satisfactorily than that previously described (4). It occurred to us that if it were possible to oxidize the carbohydrate moiety under conditions of pH which would not affect the protein component, it might be possible to obtain a chemically altered complex readily dissociable and more suitable for study of the toxic constituent. Several immunologically active polysaccharide haptens were first tested, that of Types I and XIV pneumococcus, and of Type B Friedländer bacillus. It was found in all instances that a loss or great diminution in serological activity accompanied oxidation with periodic acid. When the somatic antigen of *Sh. paradysenteriae* Type Z was treated with 0.1 M periodic acid it was observed that the carbohydrate moiety was readily oxidized and at the same time the serological activity of the complex was rapidly destroyed as

were its toxic properties, and its ability to incite specific immune bodies in experimental animals.

It was then observed that when dysentery bacilli themselves were treated with dilute periodic acid at pH 5.0 for short time intervals they no longer agglutinated readily in homologous antiserum and if the bacteria remained in contact with the reagent for an hour or more their agglutinability was markedly decreased. When examined under the microscope these cells were morphologically indistinguishable from untreated bacilli. Their loss of agglutinability was probably due to the rapid chemical alteration of the specific antigen distributed on the surface of the cell. That the toxicity of the treated bacilli was markedly reduced is evident from the tests presented in Table II. Long before the maximum consumption of periodic acid has ensued (Text-fig. 1) the toxicity of the cells appears to be greatly reduced.

Microorganisms treated with periodic acid, though diminished both in toxicity and agglutinability, are still capable of eliciting a vigorous immune response in rabbits. The antisera obtained from immunized animals agglutinate not only treated but untreated cells as well. This phenomenon is at first difficult to understand, because cells treated with periodic acid agglutinate only with difficulty and in low titre in antiserum to untreated cells. It will be recalled, however, that if the bacilli remain in contact with the reagent for prolonged periods of time, they become less efficacious in producing agglutinins not only for themselves, but for untreated cells as well. It is our belief that when dysentery bacilli are treated with periodic acid, the antigen distributed at the surface of the cell is the first to undergo oxidation. As the reagent permeates the cellular bodies the intracellular somatic antigen likewise begins to undergo oxidation. This chemical reaction is necessarily heterogeneous in nature as evidenced by the fact that extraction of treated cells with aqueous pyridine yields a soluble material which is itself heterogeneous. This extraction product can be fractionated by means of acetone to yield a component the nitrogen and phosphorus content of which is comparable to that of the unaltered somatic antigen. Yet this substance can certainly not be regarded as pure. It must necessarily be constituted of a mixture of molecular species some of which have been greatly altered by the reagent, while others, because of secondary chemical combinations with the susceptible hydroxyl groups of the carbohydrate moiety, have undergone but little chemical change. That this interpretation has some basis in fact is evident from the results of the cross-absorption tests presented in Table VIII. Here it can be seen that at least two kinds of antibodies are elicited by the fraction obtained from treated bacilli, some directed against the oxidized antigen which cannot be absorbed by untreated cells, and others which are not absorbed by treated cells, and which are specific for the chemically intact antigen. Thus it must be assumed that the product of extraction derived from

the bacilli treated for 40 minutes with periodic acid, is comprised of molecules of somatic antigen the carbohydrate moiety of which has suffered varying degrees of chemical alteration. That these chemically altered complexes are capable of eliciting immune bodies is obvious, and that the specificity of the immune response has been changed is likewise apparent, but whether periodic acid affects the protein constituent is not known at the present time. Nor has this study shed much light upon the nature of the toxic component of dysentery bacilli beyond the fact that there is an effective way of detoxifying the bacilli without destroying their antigenic efficacy.

SUMMARY

A method for detoxifying Type Z and sp. Newcastle *Sh. paradysenteriae* (Flexner) has been described. This procedure involves exposing the bacilli at pH 5.0 to 0.01 M periodic acid. Microorganisms treated with this reagent for an appropriate time interval lose approximately 90 per cent of their toxicity, yet they are capable of eliciting in experimental animals antibodies effective against the unaltered organisms.

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EXPLANATION OF PLATE 31

These photographs were made by Mr. Joseph B. Haulenbeek.

FIG. 1. Skin reactions in rabbits induced by the somatic antigen of *Sh. paradysenteriae* Type Z (a) and its product of oxidation (b). Natural size. The small dark spot in the middle lesion shown in (b) was caused by an area of skin pigmentation present before the injection was given.

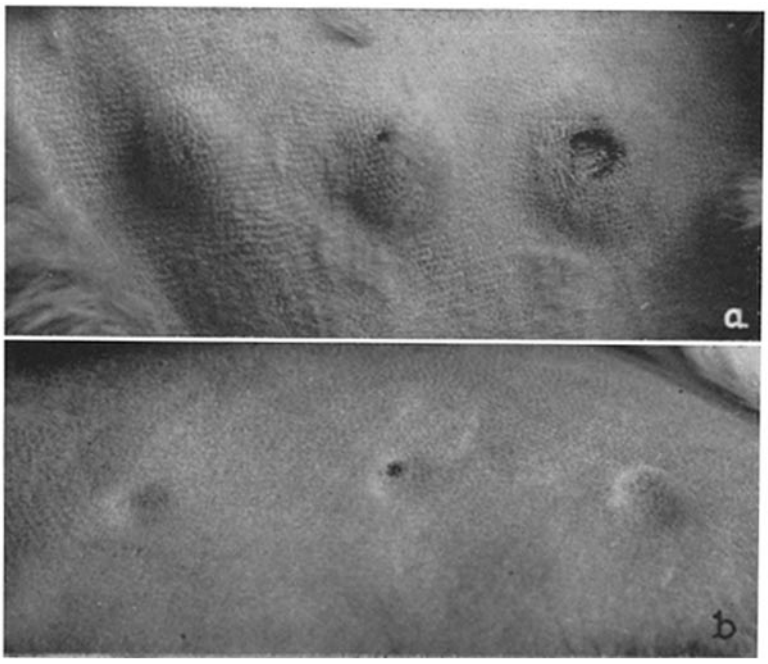

FIG. 1