ANTIGENIC PROPERTIES OF L-FORMS OF *Salmonella typhosa*

G. Ya. Kagan, E. I. Koptelova, and A. N. Bitkova
Division of General Medical Microbiology (Scientific Director, Active Member AMN SSSR V. D. Timakov), Academician N. F. Gamaleya Institute of Epidemiology and Microbiology (Director, Professor O. V. Baroyan) of the AMN SSSR, Moscow
(Presented by Active Member AMN SSSR V. D. Timakov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 55, No. 1, pp. 74-77, January 1963
Original article submitted January 16, 1962

Details of the antigenic structure of L-forms of *Streptobacillus moniliformis*, *Proteus vulgaris*, *S. typhosa*, and *Streptococcus haemolyticus* have recently been published [1-8]. These show that in the course of the L-transformation there is a partial loss of antigens of the original species of bacteria, resulting from blocking of the synthesis of individual chemical components of the cell wall, although the antigenic relationships between the L-forms and the initial bacterial form are not entirely lost. The question of the formation of new antigens, specific for L-forms and not present in the original bacterial cultures, in the course of the L-transformation remains unanswered.

During the study of the antigenic structure of the L-forms of certain pathogenic species of bacteria, we became interested in the problem of the presence of antigens in L-forms common to the original bacterial culture, and also specific, and not found in the original culture. The material used in the present investigation was a stabilized culture of L-forms of *S. typhosa* (L-152) and an initial strain of *S. typhosa* (No. 5606).

EXPERIMENTAL METHOD

A stabilized culture of L-forms of *S. typhosa*, kept for over 7 years in laboratory conditions and having lost its capacity for reversion, was seeded into a liquid nutrient medium consisting of atryptic digest of ox heart, 10% yeast extract, penicillin (500 units/ml) and 10% normal horse serum. The culture of L-forms grew in the course of 7 days in the form of a massive residue, consisting of L colonies of types 3A and 3B. They contained all the specific microstructures of L-forms: spherical and vacuolated bodies, and submicroscopic granular elements. The suspension of L-forms in physiological saline was thrice washed to remove the medium by centrifugation (3000 rpm, 45 min); the washed residue was carefully ground in a mortar, resuspended in physiological saline, and standardized in relation to the optical standard of the State Control Institute. This suspension was used for immunization of rabbits and also for production of antigen. A suspension of the bacterial culture was obtained in the same way.

The rabbits were immunized over a period of 6 weeks in accordance with the following program: 1st cycle – intravenous injection of 0.5 ml (density of suspension 10^9/ml) daily for 4 days followed by an interval of 3 days; 2nd cycle – the same with a dose of 1 ml; 3rd cycle – 1.5 ml; 4th cycle – 2 ml; 5th cycle – 2.5 ml; 6th cycle – 3 ml. Blood was taken 10-12 days after the last injection.

Serological investigations were carried out by means of the agglutination reaction, the ring precipitation test, and precipitation in jelly. For the agglutination reaction we used suspensions of bacteria and L-forms washed in physiological saline. As precipitinogen we used antigens prepared by Grassier's method (frozen ten times to –10° and thawed to 37°, density of suspension 4 billion in 1 ml of distilled water), and also Boiven's complete antigen. The results of the precipitation reaction with these two antigens were compatible. The agglutination reaction with the L-form was carried out by Klieneberger-Nobel's method [5].

The chemical composition of the bacterial and L-forms was studied by determination of the total nitrogen by the micro-Kjeldahl method, total phosphorus by the Fiske-Subbarow method, reducing substances by the Hagedorn-Jensen method, nucleic acids by the method of Schmidt and Thannhauser, DNA by Diesche's method, and glucosamine by the method of Elson and Morgan.

Antigenic complexes were produced by Boiven's method from the bacterial culture and the culture of L-forms, and these were then fractionated with a 0.1 N solution of acetic acid. As a result of fractionation, protein and polysaccharide fractions were obtained, and the amino-acid and carbohydrate compositions of these fractions were studied.

by descending distributive paper chromatography. For estimation of the amino-acid composition, the preservations were hydrolyzed with 6N HCl for 36 h, filtered, and the filtrates were applied to chromatograms; as solvent we used a butanol–acetic acid–water mixture, and this was passed 3 or 4 times through the chromatogram.

The carbohydrate composition was determined after hydrolysis with 0.1 N H_2SO_4 for 6 hr on a water bath, neutralization with $Ba(OH)_2$, centrifugation, and washing the residue with hot water, evaporation to dryness, and precipitation twice with alcohol. The residue was then dissolved in water and applied to the chromatogram.

EXPERIMENTAL RESULTS

The results of the reaction of agglutination of antisera to the L-forms with the original bacterial cultures demonstrated that stabilized cultures of L-forms retain their species specificity. Antiserum to L-forms of S. typhosa did not contain agglutinins against a culture of Proteus vulgaris, and likewise, antiserum to L-forms of Proteus vulgaris did not contain agglutinins against a culture of S. typhosa.

Stabilized cultures of L-forms preserved their antigenic links with the original bacterial culutures. For instance, antiserum to L-form of S. typhosa agglutinated the original typhoid culture in a dilution of 1:320, and antiserum to L-form of Proteus vulgaris agglutinated the bacterial form in a dilution of 1:160. In the course of L-transformation and subsequent stabilization of the L-cultures, most of the H-antigen and some of the O-antigen were lost; antiserum to the L-forms agglutinated a culture of S. typhosa H-901 in a dilution of 1:20, and a culture of O-901in a dilution of 1:320.

The culture of the L-forms was almost incapable of adsorbing antibodies to the original culture of S. typhosa, although they could be extracted completely from serum against L-forms by adsorption with the bacterial form.

In Fig. 1 we give the results of a study of the antigenic structure of the L-forms by means of the precipitation reaction in jelly. The central wells of dishes Nos. 1 and 2 contained antiserum to the L-forms, the peripheral wells of dish No. 1 contained antigen obtained by alternate freezing and thawing from L-forms of S. typhosa, and the peripheral wells of dish No. 2, antigen from bacterial forms. Comparison of the results of precipitation in dishes Nos. 1 and 2 indicates that the reaction between antigen from the L-forms and L-antiserum took place more intensively than when antigen from the original culture was used.

The central wells of dishes Nos. 3 and 4 contained antiserum to the L-forms adsorbed by a culture of S. typhosa, and the peripheral wells of dish No. 4 contained antigen of the bacterial culture. The absence of precipitation in dish No. 4 demonstrates that adsorption of typhoid antibodies was complete, while the marked precipitation ring in dish No. 3 demonstrates that stabilized L-forms of S. typhosa contained an additional antigenic component, not present in the original culture. The central wells of dishes Nos. 5 and 6 contained antiserum to the bacterial form of S. typhosa, and the peripheral wells of dishes 5 and 6 contained homologous antigen and antigen of L-forms respectively. The results of the experiment in dishes Nos. 5 and 6 demonstrate that the antiserum of the bacterial forms contained hardly any precipitins against the L-forms. The ring precipitation test gave similar results.

The change in the antigenic structure of the typhoid bacteria in the course of L-transformation was accompanied by significant changes in their chemical composition. In the L-forms there was a sharp fall in the content of the total nitrogen, reducing substances, glucosamine, and nucleic acids; the RNA/DNA ratio was only half as great as in the original typhoid culture; the lipid content was considerably increased. The yield of Boiven's complete antigen from the L-forms was only half that from the original culture of S. typhosa, and the content of total phosphorus and reducing substances in the complete antigen was also reduced.

The chromatographic analysis of the amino-acid composition of the protein fractions of the complete antigens from the L-forms and the original bacterial culture revealed no significant differences between them. During the chromatographic analysis of the polysaccharide fractions of the complete antigens (Fig. 2), it was noted that the L-forms had lost rhamnose and an unidentified sugar, present in the original culture of S. typhosa, and had gained an unidentified sugar absent in the original culture. The differences in the chemical composition of the L-forms and bacterial form demonstrate the complexity of the processes of biological reconstruction in the course of the L-transformation. The changes taking place most probably affect not only the process of synthesis of the cell walls (loss of rhamnose, decrease in glucosamine), but also the synthesis of the cytoplasm and the nuclear apparatus of the cell (decrease in total nitrogen, phosphorus, nucleic acids, and reducing substances, increased lipid content).

The comparison of the character of the changes in the antigenic structure of the L-forms and their chemical composition demonstrates that, besides a loss of some of the O-antigens and of most of the H-antigens, there is a loss

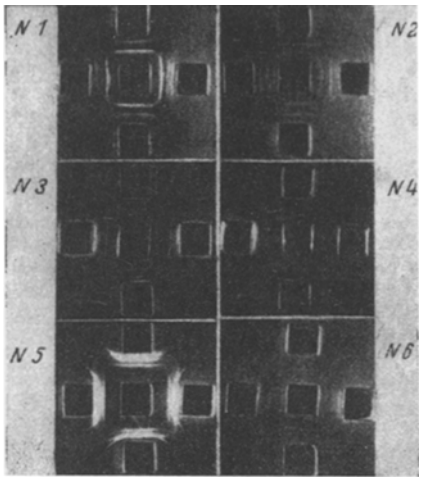

Fig. 1. Details of the antigenic structure of L-forms of S. typhosa. No. 1 – antiserum and Grassier's antigen from L-forms of S. typhosa; No. 2 – antiserum to L-forms of S. typhosa, Grassier's antigen from bacterial forms; No. 3 – antiserum to L-forms adsorbed by bacterial form, Grassier's antigen from L-forms; No. 4 – control of completeness of adsorption; No. 5 – antiserum to bacterial forms of S. typhosa, Grassier's antigen from S. typhosa; No. 6 – antiserum to bacterial forms of S. typhosa, Grassier's antigen from L-forms.

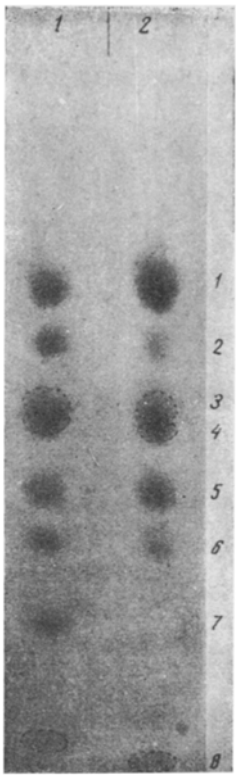

Fig. 2. Chromatogram of carbohydrate composition. 1) Galactose; 2) glucose; 3) mannose; 4) arabinose; 5) xylose; 6) ribose; 7) rhamnose; 8) unidentified sugar.

of rhamnose and a decrease in the content of reducing substances and glucosamine. The formation of an additional antigenic component in the process of L-transformation coincides with the appearance of an additional, unidentified sugar, not present in the original culture of S. typhosa.

SUMMARY

The stabilized culture of the L-form of S. typhosa retains its species specificity, largely loses the H-antigen and to a lesser degree the O-antigen. The precipitation reaction in gel has aided in detecting an additional antigenic component, absent in the initial culture in the antigenic complex obtained from the L-form culture by alternating freezing and thawing.

The changes in the antigenic structure during the process of L-transformation are accompanied by significant changes in the chemical composition. Apparently these changes concern not only the process of biosynthesis of cellular walls (as for instance the loss of rhamnose, glucosamine reduction, etc.) but also the biosynthesis of the cytoplasm and of the nuclear apparatus of the cell (reduction of the total nitrogen, phosphorus and nucleic acid content). The formation of an additional antigenic component during the process of L-transformation coincides with the appearance of an additional nonidentified sugar, which was absent in the initial typhoid culture.

LITERATURE CITED

1. V. D. Timakov, G. Ya. Kagan, and V. S. Levashev, Antibiotiki, 4, 214 (1958).
2. L. Dienes, H. J. Weinberger, and S. Madof, J. Bact., 1950, v. 59, p. 755.
3. D. J. Edward and W. A. Fitzgerald, J. Path. Bact., 1954, v. 68, p. 23.
4. E. Friemer, R. M. Krause, and M. McCarti, J. exp. Med. v. 110, p. 853.
5. E. Klieneberger-Nobel, Zbl. Bakt. I Abt. Orig., 1958, Bd. 75, S. 376.
6. Z. Loekiewiez, E. Hulanca, and H. Weinrauder, Acta mikrob. Pol., No. 6, p. 311.
7. J. Poetschke, L. Killisch, et al., Z. Immun. Forsch., Bd. 111, S. 406.
8. J. Sharp, S. W. Hijman, and D. Dienes, J. exp. Med., 1957, v. 105, p. 153.