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Antigenic Relationship of Streptococcal Diphosphopyridine Nucleotidases.* (28179)

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Antibodies capable of specifically inhibiting the enzymatic activity of streptococcal diphosphopyridine nucleotidase (DPNase) are found in the sera of humans and experimental animals(1,2). These occur with other streptococcal antibodies and increase in the serum in response to streptococcal infection(1). Bernhard and Stollerman(2) suggest that the anti-streptococcal DPNase antibody (ASDA) titration be used in conjunction with anti-streptolysin O titrations to increase the accuracy of diagnosis of antecedent streptococcal infections. These observations imply an antigenic identity of streptococcal DPNase regardless of source. This investigation tests the validity of the assay by determining the antigenic relationship of a number of DPNases from various groups and types of hemolytic streptococci.

Materials and methods. The following strains of hemolytic streptococci were employed in this study: *Streptococcus pyogenes* Group A, types 4-366, 4-3799, 12, 12-350, 12-H8, 12-Witcher, 14, 19, and 28; Group C strains C74, H46a, and 2927; and Group G strain 2986. The medium was that of Todd and Hewitt(3). On occasion, streptococcal lesion extract (SLE) was used as DPNase source prepared by the method of Schwab, Watson, and Cromartie(4). DPNase activity was determined by the method of Carlson, *et al.*(5) using the supernatant fluids from 24-hour cultures which had been centrifuged at 3,000 rev/min for 15 minutes. The unit of DPNase activity was defined as the amount of enzyme that destroys 0.01 micromole of diphosphopyridine nucleotide‡ (DPN) in 7½ minutes at 37°C.

ASDA determinations were done by the method of Kellner, Freeman, and Carlson(1). One unit of ASDA activity is the amount of antibody neutralizing 100 units of DPNase. In cross-neutralization assays, pre-titrated antisera were used at a concentration of approximately one ASDA unit; this permitted the titration of a single enzyme against several pre-titrated antisera reducing the error in the assay.

Immunization of American Dutch rabbits consisted of injecting 3 ml aliquots of the enzyme source, Todd-Hewitt culture filtrate or SLE, on alternate days. The first 3 injections were given intravenously followed by the intradermal route. When a significant Arthus

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TABLE I. Effect of Antigenic Dose on ASDA Response in Rabbits.

DPNase	Source	DPNase, units/ml	Total DPNase inj, units	ASDA, units/ml
TH*	4	3,200	44,800	80
"	12-W	2,700	37,800	93
"	28	4,000	56,000	59
"	C-74	270	3,780	10
"	G-2986	1,200	14,800	17
SLE†	4	490	8,840	2
"	12-H8	380	6,080	1
"	14	0	0	0
"	19	0	0	0

* Todd-Hewitt broth filtrate.
† Streptococcal lesion extract.

reaction appeared, usually after the seventh injection, the animals were bled 7 days after the last injection. Each antiserum is a pool from several animals.

Results. Table I shows that in rabbits, the amount of ASDA produced is roughly proportional to the total amount of enzyme injected. Group A streptococci, types 14 and 19, not capable of producing DPNase *in vitro*, failed to produce detectable quantities of the enzyme when cultivated in an *in vivo* environment.

A serum from a patient with rheumatic fever was made available by Dr. Lewis Wannamaker, Dept. of Pediatrics, Univ. of Minnesota; this serum was titrated against DPNases from several streptococcal groups and types. Results given in Table II show that human serum neutralizes these streptococcal DPNases with equal facility. Although this suggests antigenic relationship between all of the DPNases tested, it is possible that patients repeatedly infected with streptococci become exposed to and develop antibodies to a number of antigenically distinct enzymes including those tested here. Results given in Table III show the antigenic relationship of these enzymes. Cross-neutralization tests, using antibodies induced specifically in rabbits with enzymes from various types and groups of streptococci, fail to show any type or group specificity. Antisera prepared from DPNase-negative types 14 and 19 were included to show that the inhibition observed is not induced by nonspecific factors in the extracts. It also serves as a sensitive indicator for the presence of small quantities of enzymes.

Discussion. In contrast to streptococcal hyaluronidases which are group specific(6), the DPNases of these organisms are antigenically related regardless of the types or groups from which the enzymes are derived. This suggests that the antigenic determinant of DPNase is more closely associated with the active enzyme site while that in hyaluronidase may be to a less specific portion of the enzyme molecule.

TABLE III. Antigenic Relationship of DPNases Determined by ASDA Cross-Neutralization Tests.

Antigen*	4	12W	14	19	28	C-74	G-2986
4	10†	100	0	0	75	12	23
4-3799	6	112	0	0	48	11	18
12	10	80	0	0	44	8	15
12-350	5	88	0	0	48	9	13
12-H8	3	64	0	0	30	5	12
12-W	8	108	0	0	66	9	16
28	8	76	0	0	51	11	14
C-74	8	100	0	0	57	11	18
C-H46	10	104	0	0	57	13	23
C-2927	11	108	0	0	96	12	17
G-2986	7	80	0	0	80	13	19

* Horizontal column refers to the antigen (DPNase) used to immunize the rabbits; vertical column is the antigen (DPNase) used as enzyme source in the ASDA titration.
† ASDA units per ml of serum.

All Group A streptococci do not produce DPNases. One of these, a Group A, type 19, was an epidemic strain associated with a significant incidence of rheumatic fever. Cultivation in an *in vivo* environment, a technique used to enhance production of other streptococcal factors(7), did not induce this organism to make DPNase.

Summary. DPNases from various groups

TABLE II. ASDA Assay of a Human Serum Employing DPNases from Several Groups and Types of Hemolytic Streptococci.

	DPNase source*								
	4	12	12-350	12-H8	12-W	28	C-2927	C-74	G-2986
ASDA, units/ml	1200	1440	1440	1440	1400	1400	1280	1000	1320

* Refers to streptococcal strain used as DPNase source in ASDA titration.

and types of hemolytic streptococci are antigenically related. Group A streptococci not producing DPNase *in vitro* also failed to make detectable enzyme *in vivo*.

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Dietary Protein Levels, Vitamins, and Lactose on Depressant Effect of Sulfadrugs on Urinary and Liver Ascorbic Acid in the Rat. (28180)

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It has been reported(1) that increase in dietary protein level enhances urinary excretion and liver ascorbic acid content in the rat. Preliminary observations on the depressant effect of sulfaguanidine in low protein diets and its counteraction on 50% protein diets were also recorded. This report concerns an investigation of the effects of sulfaguanidine and sulfasuccidine in relation to dietary protein levels and supplementation with lactose and certain vitamins.

Experimental materials and methods. As in the earlier investigations, these observations were made on adult male albino rats in the body-weight range 160-200 g. Composition of the diets and details of collection of urine were as described previously. The basal diet provided Vit. A and D and thiamine, riboflavin, pyridoxine, pantothenate, niacin and folic acid. In testing the effect of some of the other vitamins they were administered either orally or intraperitoneally. In the final test to ascertain the influence of supplements of all the vitamins omitted from the basal diet the vitaminized starch used at 1% level in the diet contained: 0.5 g menadione (sodium bisulfite), 0.5 g thiamine, 1.0 g riboflavin, 0.4 g pyridoxine, 4.0 g calcium pantothenate, 4.0 g niacin, 0.05 g folic acid, 0.02 g biotin, 0.002 g Vit. B_{12}, 200.0 g choline chloride and 25.0 g inositol per kilo, and the oil soluble vitamins were provided by feeding orally one drop of a suitably diluted stock solution of Vit. A acetate, calciferol and tocopheryl acetate in refined peanut oil so as to provide 20, 10 and 1 I.U. of Vit. A, D and E respectively to each rat daily. In this experiment the salt mixture originally used was also supplemented with 40 mg zinc carbonate per kilo to make up any deficiency of the diet in zinc.

Total ascorbic acid in urine and tissue was estimated by the Roe and Kuether method (2).

Results. 1. *Influence of dietary protein levels.* Data in Table I show results of ingestion of sulfaguanidine and sulfasuccidine at 2-2.5 and 4% levels in diets containing different amounts of protein. The findings fully confirm previous observations concerning the depressant effect of the sulfadrugs on urinary and liver ascorbic acid levels with low protein diets and the counteracting influence of higher levels of protein in the diet, the effect being completely nullified by 50% protein in the diet. The results obtained with the 2 sulfadrugs and at the 2 levels were similar. In the present series of experiments the effect of the sulfadrugs on liver ascorbic acid was not as pronounced as in the previous study(1), except in animals receiving protein-free diets.

Urinary excretions observed in these experiments corroborate the earlier finding(1) that prolonged ingestion, in contrast to short-term feeding, tends to equalize the differences