ENZYME PATTERNS OF VIRULENT NEISSERIA GONORRHOEAE

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CERTAIN ENZYMES of nonvirulent stock cultures of *Neisseria gonorrhoeae* have been investigated (*1*), but no reports of enzyme studies of virulent isolates of this organism are available.

In an investigation at the Venereal Disease Research Laboratory, virulence of *N. gonorrhoeae* was retained by using fresh isolates and a rapid cultivation procedure. A reduced diphosphopyridine nucleotide oxidase system, a lactic dehydrogenase, a glutamic dehydrogenase, an alcohol dehydrogenase, and an acetate oxidase system were studied. All enzyme assays were done within the period of virulence, and enzyme activities were compared with relative resistance of the isolates to penicillin (*2*). A report of this study includes general observations on the enzymology of *N. gonorrhoeae* (*1*).

The procedure for rapid cultivation of *N. gonorrhoeae* is described below.

Methods

Preparation of liquid medium. The liquid medium described by Tauber and Russell (*3*), which has been used by the Venereal Disease Experimental Laboratory for the cultivation of *N. gonorrhoeae* for several years, has the following composition:

	Grams per liter
Soluble starch	0.5
Yeast extract (BBL or Difco)	1
Proteose peptone No. 3 (Difco)	8
Na_2HPO_4	3
NaCl	5
Glucose	2

In this study the glucose was autoclaved for 15 minutes at 121° C. in 50 percent concentration and then was added to the medium, which had been autoclaved for 20 minutes at 121° C. The pH of this medium was 7.20.

The pure isolates were grown for 1 or 2 days on slants, for 2 days in test tube cultures, and for 3 to 4 days in Erlenmeyer flasks at 36° C. The cells were collected in a Servall centrifuge, washed twice with 0.85 percent sodium chloride solution, and once with distilled water. Reduced diphosphopyridine nucleotide oxidase, being a relatively labile enzyme, was assayed immediately after harvesting. The remainder of the cells were stored frozen at −20° C.

Slant cultures. Slant cultures were prepared, using Bacto Proteose No. 3 agar and hemoglobin (*2*). Penicillin was omitted from the medium. Sterile dextrose solution was added to a final concentration of 1 percent. Both solutions were placed separately in the autoclaved Proteose No. 3 agar at 56° C. Hemoglobin solution at 56° C. was added to the Proteose agar solution, mixed, and aseptically dispensed.

Liquid medium cultures. From slant cultures transfers were made to test tubes containing 6 ml. portions of liquid medium. From these "seeding" cultures transfers were made to 500 ml. Erlenmeyer flasks containing 150 ml. portions of liquid medium. The cultures were placed in large desiccators and grown in an atmosphere of CO_2 (candle method).

Enzyme assays. Methods for determining the several enzymes, except for glutamic dehy-

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drogenase, using the Thunberg technique and color-forming triphenyltetrazolium chloride as the electron acceptor, have been previously described (*1*). For the determination of glutamic dehydrogenase, the following method was employed.

Eighty milligrams of wet bacteria was suspended in 4 ml. of distilled water and dispersed in a glass homogenizer. In Thunberg tubes were placed 1 ml. portions of the material, 1 ml. of 0.05 M glycine buffer of pH 9.1, 0.5 ml. of glutamic acid solution (0.2 M) adjusted to pH 8.0, and 0.3 ml. (2 mg.) of 2,3,5, triphenyltetrazolium chloride. In side arm was placed 0.2 ml. (0.1 mg.) of diphosphopyridine nucleotide in 0.01 M phosphate buffer of pH 7.5. The final volume was 3 ml. Tubes were evacuated and placed in a water bath at 37° C. After 10 minutes, the contents of the side arm were added to the tubes. After 30 minutes, the reaction was stopped by placing the tubes in an ice bath. Formazan was extracted with 7 ml. of cold acetone. The acetone extracts were centrifuged and the optical densities of the supernatants were measured spectrophotometrically at 520 μ (*1*). Controls were done on all experiments.

Results

In column 2 of the table are recorded values for penicillin resistance for 30 virulent *Neisseria gonorrhoeae* isolates. The isolation procedure is described in reference 2. Enzyme activities are expressed as micrograms (μg.) of formazan per 100 μg. of nitrogen. Formazan produced by reduced diphosphopyridine nucleo-

Enzymes of virulent Neisseria gonorrhoeae

Isolate No.	Resistance to penicillin [1]	DPNH oxidase [2]	Lactic DH [2]	Glutamic DH [2]	Alcohol DH [3]
Group 1: [4]					
655	0. 05	240	135	0	3
701	. 01	269	297	0	0
683	. 05	306	228	26	0
656	. 05	337	175	0	0
662	. 05	361	295	0	0
GLF–62	. 05	371	379	111	4
654	. 05	375	188	53	0
688	. 01	394	197	41	4
633	. 01	406	243	36	0
676	. 05	412	284	53	0
708	. 01	589	203	44	4
685	. 01	613	231	61	1
661	. 01	626	231	0	2
704	. 01	627	347	82	0
658	. 01	686	196	41	3
Group 2: [5]					
634	0. 20	704	324	48	6
659	. 25	731	230	0	4
657	. 15	744	296	54	6
636	. 01	776	197	21	0
635	. 10	776	235	51	0
617	. 01	910	359	6	1
687	. 01	933	161	100	10
613	. 05	983	303	40	3
652	. 15	1, 037	327	43	1
709	. 10	1, 054	262	95	0
590	. 05	1, 063	396	49	6
707	. 05	1, 078	313	67	0
706	. 05	1, 105	226	68	0
660	. 01	1, 194	189	73	1
678	. 01	1, 245	224	51	3

[1] Units of penicillin per milliliter.
[2] Micrograms of formazan per 100 μg. of nitrogen.
[3] Degree of reaction arbitrarily graded from 0 to 10.
[4] All isolates sensitive to penicillin.
[5] 40 percent of isolates resistant to penicillin; high incidence of reduced diphosphopyridine nucleotide.

NOTE: DPNH, reduced diphosphopyridine nucleotide; DH, dehydrogenase.

tide oxidase varied between 240 and 1,245 μg.; for lactic dehydrogenase between 135 and 396; for glutamic dehydrogenase 0 and 111 μg. and for alcohol dehydrogenase between 0 and 10+. All isolates contained a cyanide sensitive aerobic cysteine oxidase and a cysteine desulfhydrase, and most contained small quantities of an acetate oxidase system.

The 30 isolates may be divided into 2 groups. All of the isolates of the first group were sensitive to penicillin, having a range of sensitivity between 0.01–0.05 unit per milliliter. This group had the lowest formazan values for reduced diphosphopyridine nucleotide oxidase (240–686 per 100 μg. of nitrogen) or 100 percent correlation between high sensitivity to penicillin and low reduced diphosphopyridine nucleotide oxidase values. In the second group of the isolates, reduced diphosphopyridine nucleotide was high (704–1,245), and the resistance to penicillin was high in 40 percent of these isolates (0.10–0.25 unit per milliliter).

There were no appreciable quantitative changes in the activities of the respective enzymes after 12 transfers using the liquid medium. Transfers were made every 2 days. Storing of the bacteria at −20° C. did not affect the enzymes, nor did repeated freezing and thawing, except for reduced diphosphopyridine nucleotide oxidase, which lost much activity unless assayed immediately after harvesting. There was only partial correlation between the enzyme patterns of virulent isolates of *N. gonorrhoeae* and the enzyme patterns of nonvirulent isolates (*1*).

Summary

In an investigation of 7 enzymes of 30 virulent isolates of *Neisseria gonorrhoeae*, only 1 enzyme, reduced diphosphopyridine nucleotide oxidase, showed extensive variations among the various isolates studied. In enzymes with the highest reduced diphosphopyridine nucleotide oxidase values, 40 percent showed high resistance to penicillin.

REFERENCES

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(*2*) U.S. Public Health Service: Gonococcus. Procedures for isolation and identification. PHS Publication No. 499. U.S. Government Printing Office, Washington, D.C., 1961.

(*3*) Tauber, H., and Russell, H.: General methods for the isolation of endotoxins. Exper. Med. & Surg. 19: 162–169, June–September 1961.

Work Therapy Program in Baltimore

An experimental "on the job" work therapy program for psychiatrically disabled patients is being conducted in Baltimore under nonmedical supervision by the Metropolitan Baltimore chapter of the National Association for Mental Health, Inc.

The trainees work 30 hours a week at the chapter's headquarters. They serve as phone receptionists, news release writers, address verifiers, label pasters, mail clerks, dictaphone operators, stenographers, proofreaders, or multilith operators. One community volunteer does the same type of work with every three trainees.

Of the 27 individuals who have rotated through the program since October 1960, two-thirds have obtained jobs, entered specialized schools of training, or have been able to leave the hospital and return to the community. One-fourth of the trainees were still hospitalized when they entered the program.