

greater in the tetanus than in the single twitches at a rate of 60 per minute.

Owing to variations in the amount of work performed by individual muscles, it was possible to construct curves by plotting lactic acid or hexosephosphate against work. Lactic acid was proportional to the work both in single twitches and in tetani and the curves intersected zero work at the basal value of lactic acid as determined experimentally. Hexosephosphate was not proportional to the work; it rose rapidly with small amounts of work and less so as more work was produced, both in single twitches and in tetani.

Oxidative removal of lactic acid and hexosephosphate was excluded by performing some of the experiments in nitrogen. The difference in time between slow and fast stimulation (5 minutes) is too small to allow hexosephosphate to disappear, as shown in experiments in which the rapidly stimulated muscle was fixed 5 minutes after the end of stimulation. Hexosephosphate and lactic acid account for most of the glycogen broken down during contraction. The greater increase in hexosephosphate with rapid stimulation represents an extra breakdown of glycogen, while the relation of hexosephosphate to work suggests that as work is increased proportionately less glycogen is broken down.

## 7312 P

### Antigenic Relationships of Diphtheria Bacilli and Derived Variants.

GEORGE T. RICH AND LLOYD R. JONES. (Introduced by Moyer S. Fleisher.)

*From the Department of Bacteriology and Hygiene, St. Louis University School of Medicine.*

The antigenic relationships between diphtheria bacilli and certain derived variants have been investigated by means of the complement fixation reaction.

Two strains of virulent diphtheria bacilli, *No. 19* and *Park No. 18* and avirulent variant strains derived from these strains were used in the experiments, and in addition, a strain of *C. hofmanni*.

Two of the variant strains, *Park No. 8 phage a* and *Park No. 8 phage b*, were derived from young cultures of *Park No. 8* to which specific bacteriophage had been added. Four of the variant strains were derived in various ways from the *No. 19* culture and are designated as follows: *19 'antisera'*, derived from culture in broth con-

taining homologous antiserum, 19 'broth 45°C.', from broth culture incubated at 45°C.; 19 'LiCl', from culture in broth containing 0.5% lithium chloride; 19 'phenol', from culture in broth containing 0.1% phenol.

These variant organisms were found to be stable in type, grew very slowly in broth, and were completely lacking in fermentative ability. The variant colony was much smaller than the typical colony of diphtheria colony. Microscopically, the variant organisms were hardly distinguishable from the parent organisms. Usually they stained more solidly, but many barred and granular forms were observed.

Sera taken from some of the rabbits before immunization failed to fix complement except when large amounts of serum were used. The course of immunization consists of 8 to 10 injections of heat-killed organisms. The sera obtained were inactivated by heating them at 60-62°C. for 30 minutes.

The sera were used in 4 amounts: 0.025, 0.012, 0.006, and 0.003 cc. All antigens were used in such an amount that 4 times that amount was not anticomplementary. The smallest amount of serum giving a strong reaction with the homologous antigen was used as a base and the reactions in this amount with other antigens is reported in the tabulation.

TABLE I.

		Antiserum:							
Antigen	Park No. 8	Park No. 8, 'phage a'	Park No. 8, 'phage b'	19	19 'broth, 45° C.'	19 'antiserum'	19 'LiCl'	19 'phenol'	C. hofmanni
Park No. 8	+4	+3	—	+2	—	+3	+2	+2	—
Park No. 8 'phage a'	—	+4	—	+2	—	—	—	—	—
Park No. 8 'phage b'	+2	+3	+4	—	+1	+2	—	+2	—
19	—	—	—	+4	+1	+1	—	—	—
19 'broth 45° C.'	—	+3	—	+4	+4	—	—	—	—
19 'antiserum'	—	—	—	+4	—	+4	—	—	—
19 'LiCl'	—	—	—	—	—	—	+4	—	—
19 'phenol'	—	+2	—	—	+1	+2	—	+4	—
C. hofmanni	—	—	—	—	—	—	—	—	+4

*Park No. 8* and *No. 19*, the virulent parent organisms are antigenically dissimilar. *Park No. 8* differs from both of its variants. *Park No. 8 phage a* antiserum reacts with all *Park No. 8* antigens, but the *phage a* antigen does not have the reciprocal relationship

with *Park No. 8* antisera. *Park No. 8 phage b* antiserum reacts only with the specific antigen; however, the *phage b* antigen has a wide reactivity with both *Park No. 8* and heterologous antisera.

The *No. 19* immune serum has a wider range of reactions than *Park No. 8* antiserum. It has strong reactions with 2 of its variants, *19 'antiserum'* and *19 'broth 45°C'*, but does not react with the remaining 2. It also reacts to a slighter extent with the heterologous *Park No. 8* and *Park No. 8 phage a* antigens. It is interesting to note that its reaction with *19 'broth 45°C'*, was stronger than with the homologous *No. 19* antigen although the table does not indicate this reaction. The *No. 19* antigen reacted less intensely in the reciprocal relationships. *19 'LiCl'* and *19 'phenol'* bear no antigenic relationship to the *No. 19* strain. On the other hand their antisera react with *Park No. 8* antigen.

A multiplicity of antigenic factors may account for the diversity in antigenic variation amongst the variants. Dissociation seems to alter the antigenic structure of a single strain in a number of possible different ways. Variants from the same parent strain, though similar in morphological and cultural characteristics and derived in a similar manner, may differ in antigenic nature. The antigenic character of a variant may be so altered that it will react with heterologous strains or variants which will not react with the parent strain.

The literature affords conflicting reports regarding the antigenic relationship of the Hofmann bacillus to the diphtheria bacillus. Our results afford no indication of a relationship.

### 7313 P

#### Action of Veratrine on Medullated Nerve.

FRANCIS O. SCHMITT, HELEN TREDWAY GRAHAM AND OTTO H. A. SCHMITT.

*From the Departments of Zoology and of Pharmacology, Washington University, St. Louis.*

Fromherz<sup>1</sup> has recently investigated the effect of veratrine and other drugs upon the electrical response of medullated nerve, using a moving coil galvanometer as a measuring instrument. He detects little difference in response between normal and veratrinized nerve, if the nerve is suspended in oxygen throughout. If, how-

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<sup>1</sup> Fromherz, H., *J. Physiol.*, 1933, **79**, 67.