POSSIBLE RELATIONSHIP BETWEEN CHOLINE-DEPENDENCE AND VIRULENCE OF A STRAIN OF PNEUMOCOCCI

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In the course of a study designed to elucidate the mechanism of action of hemicholinium no. 3 (Schueler, 1955, 1960), we have made an observation which seems to be of sufficient interest to warrant publishing a note on it at this time. This observation suggests that the pathogenicity in mice of Diplococcus pneumoniae may be associated with the ability (or inability) of this organism to synthesize choline.

The requirement of choline in the growth medium for a number of strains of virulent pneumococci was studied in detail by Badger (1944), and this requirement is by now well established (Hoeprich, 1957). However, on reconstituting a lyophilized culture of the CHA strain of type III pneumococcus (obtained from the American Type Culture Collection, Washington, D.C.), we were at first unable to demonstrate this requirement, since growth was as abundant in the absence of choline as it was in its presence. The only difference between the conditions of our experiments and those of Badger was that in the latter the culture had been passed through mice three times weekly for a period of several years, and accordingly we decided to initiate a similar program in an effort to induce choline-dependence.

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The medium was prepared according to Gibert (1944), except that the vitamin-free casein hydrolysate used was obtained from a commercial source (Nutritional Biochemicals Corporation) and was used in a concentration of 200 ml. per liter of medium. Also, pyridoxine HCl was added to the medium in a concentration of 1 mgm./liter. Swiss albino mice, weighing apx. 20 grams each, were used as the host animals.

Growth was initiated in a veal infusion broth enriched with 2% sterile defibrinated rabbit blood. After incubating 24 hours at 37° C., one ml. of the culture was diluted 1:1,000,000 with sterile saline, and 1 ml. of the resulting suspension injected intraperitoneally into each of a group of mice. As soon as the first mouse had died, a second mouse was anesthetized with ether, and, using aseptic technique throughout, 1 ml. of sterile saline was injected into its peritoneal cavity. One ml. of the peritoneal wash was then aspirated and diluted 1:1,000,000 with sterile saline, and the resulting suspension used to infect a new group of mice. A 1:1000 dilution was used to inoculate the growth medium, one tube containing choline (as the chloride, 6 mgm/liter) and the other being choline-free. The inoculated tubes were incubated at 37° C., for approximately 24 hours, and turbidity resulting from growth was measured in a Klett-Summerson colorimeter equipped with a 540 mμ filter.

In the first set of mice, each injected with 1.0 ml. of the 1:1,000,000 dilution, an incubation period of up to 24 hours was required before all the mice were dead. After only three passages through the host, however, virulence had increased to such an extent that as little as 0.1 ml. of the suspension would cause death in 10 out of 10 animals within 8 hours. Microscopic examination disclosed that capsule formation increased markedly with each transfer.

Following each passage through the host, growth in the choline-free medium relative to growth in the choline-containing medium decreased, as shown by the data in table 1.

Table 1. Effect of Passage Through Mice on Growth of Pneumococci in Choline-free and Choline-containing Media

	Colorimeter reading *		Ratio a / b
	(a) choline-free	(b) with choline	
After 1 passage	272	288	0.94
After 2 passages	94	207	0.45
After 3 passages	41	190	0.22

* Uninoculated medium reads zero.

Although it is possible to interpret the results of this experiment in several ways, such as elimination by the host of non-choline-dependent mutants (cf. Smith, 1960), one conclusion that is suggested is that as the virulence of the pneumococci increases so also does their need for externally supplied choline increase. This in turn suggests, among other possibilities, that the pathogenicity of this organism may be associated with the loss of an enzyme necessary for the synthesis of choline.

As our original purpose was to obtain a choline-dependent organism for use as a pharmacological tool, we have not extended our study of the nutritional requirements of the pneumococci further than is reported here. Whether a similar phenomenon can be demonstrated with growth

factors other than choline remains to be seen.

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