of bridge formation, since cellular connection was observed end to end, end to side, or side to side. The bridge varied from 125 A (Fig. 1) to 500 A (Fig. 6) in diameter and from 1,500 to 9,000 A in length. It is perhaps significant that the longest bridge yet observed also was the thickest, suggesting that increased lengths are not attributable to stretching. Occasionally, a single dense line, 40 A wide and continuous with the outer layer of the cell wall, formed the boundaries of the bridge (Fig. 1). More frequently, the bridge appeared to be bordered by two double structures, each approximately 110 A wide and resembling the usual dense-light-dense arrangement of the cell wall (Fig. 5 and 6). It has not yet been possible to determine whether these double structures are continuous with the similar cell-wall structures; however, enough similarity exists to permit this as a tentative conclusion.

The cytoplasmic membrane appeared unbroken at the site of bridge attachment and would seemingly act as a barrier to deoxyribonucleic acid (DNA) transfer. It is possible, however, that a point of discontinuity may be present in adjacent but unobserved areas, or perhaps discontinuity of the cytoplasmic membrane is not a necessary precursor for transfer of nuclear material. In Fig. 1 we can observe that the nuclear region (arrow) of the cell (A) is near the site of bridge formation. The internal density of the bridge in Fig. 2 appears greater than that of the background and approaches that of the cytoplasm. Other bridges have a density comparable with the chromatin area of the cell.

Anderson et al. (Ann. Inst. Pasteur **93**:194, 1957), in a study of conjugation as observed in shadowed whole-cell preparations, noted that bridges are fairly strong, being difficult to break with a micropipette. Figures 3, 4, and 5 show an unusual architectural arrangement in which the bridges appear to be coiled between cells. This spiral configuration probably resulted from twisting during preparatory procedure, and, since cellular connections are still evident, serves to confirm the hypothesis of bridge durability.

Collection of statistical genetic data concerning actual recombination of the particular bacterium is under way and will be of considerable value in determining whether the structures reported here are in fact conjugatory bridges, or possibly only plasmodesmata. Further work is now in progress to clarify the following: the occurrence of recombination with this bacterium, the direct morphological evidence of DNA transfer, the mode of formation of the bridge, and the complete moiety of the bridge.

The author gratefully acknowledges the many helpful suggestions of David B. Scott and Marie U. Nylen during the course of this study and in the preparation of this manuscript; to Judith Foote especial appreciation is expressed for preparation of specimens.

DEFINED MEDIUM FOR *HAEMOPHILUS INFLUENZAE* TYPE B

HAROLD L. WOLIN

Department of Microbiology, Seton Hall College of Medicine and Dentistry, Jersey City, New Jersey

Received for publication 13 August 1962

Increased interest in the nutrition of *Haemophilus influenzae* type d has led to the development of "synthetic" media (Talmadge. and Herriott, Biochem. Biophys. Res. Commun. **2**:203, 1960; Butler, J. Gen. Microbiol. **27**:51, 1962). Our studies with *H. influenzae* type b have provided different results, which form the basis of this report.

From a modified Cohen and Wheeler (Am. J. Public Health **36**:371, 1946) complex medium, by a process of substitution and deletion, the defined medium shown in Table 1 was developed. Incubation was carried out on a rotary shaker (140 1-in. rev/min) at 37 C by placing a drop of inoculum from a preceding 24-hr growth into 20 ml of medium contained in a 125-ml Erlenmeyer flask. At least 7 to 14 serial subcultures were made in this fashion for any one experiment. Optical density was estimated with the aid of a Coleman spectrophotometer at 660 mμ. Growth was not obtained in a medium containing a mixture of free purine and pyrimidine bases, but could be

obtained when adenosine triphosphate, adenylic acid (mixed 2′ and 3′ isomers), adenosine, or a mixture of nucleosides was added. A mixture of nucleosides, including adenosine, guanosine, uridine, thymidine, and cytidine, could support growth. Omission of any single nucleoside from the mixture of bases had little or no effect on subsequent growth. Increasing the adenosine level to 7 mg per 100 ml, in the absence of any other purine or pyrimidine base, resulted in as good growth as when the mixture of nucleosides was supplied in the medium. Uridine alone was also capable of supporting growth of *H. influenzae* type b in this medium, but was inferior to adenosine. Arginine was the sole amino acid found to be essential for growth. No vitamins were found to be essential. Undefined, nutritionally complex materials added to the final medium did not improve growth, nor decrease the time required for development of total growth. Figure 1 compares the growth achieved in the defined medium to growth achieved in the complex Levinthal medium.

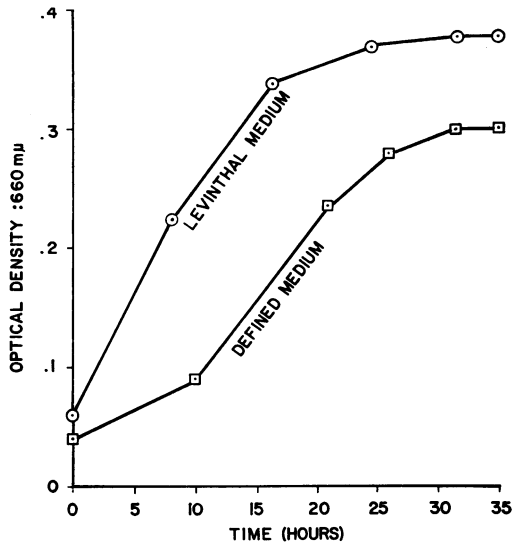

FIG. 1. *Growth of Haemophilus influenzae type b in defined medium and in Levinthal medium.*

TABLE 1. *Composition of a defined medium for a strain of Haemophilus influenzae type B*

Substance[a]	Amount	Substance	Amount
	mg/liter		*mg/liter*
Tris[b] buffer	4,340	Tween 40[c]	16
Glucose	5,000	Hemin	10
Glycine	50	$CaCl_2 \cdot 2H_2O$	10
DL-Isoleucine	280	K_2HPO_4	670
L-Lysine	290	$MgSO_4 \cdot 7H_2O$	100
L-Leucine	340	$NaHCO_3$	270
L-Cystine	50	NaCl	5,340
L-Methionine	90	Inositol	5
L-Tyrosine	220	Thiamine HCl	0.20
L-Tryptophan	50	Folic acid	0.10
L-Asparagine	250	Ca pantothenate	1.5
L-Glutamic acid	750	Biotin	0.02
L-Arginine	140	Coenzyme I[d]	1.0
Adenosine	70	pH 7.6	
Polyvinyl alcohol	600		

[a] Precipitation of medium avoided by addition of the magnesium salt first, followed by dipotassium phosphate and then calcium chloride.

[b] Tris(hydroxymethyl)aminomethane.

[c] Polyoxyethylene sorbitan monopalmitate (Atlas Powder Co.).

[d] Diphosphopyridine nucleotide (95%) obtained from Mann Laboratories and sterilized by Seitz filtration. Remainder of medium sterilized by autoclaving at 10 psi for 10 min. All medium constituents dissolved in distilled water.

A constant problem during much of our early work was caused by the sudden occurrence of poor growth of organisms after repeated passage, even in the same lot of medium, and its sudden return to normal on continued transfer. Incorporation into the medium of polyvinyl alcohol, originally contained in the medium of Talmadge and Herriott, appeared to reverse this toxic effect. Although repeated efforts to grow our strain of *H. influenzae* type b in the medium of Talmadge and Herriott were unsuccessful, the finding of an absolute requirement for arginine, and the need for the presence of at least a single nucleoside, were similar to their observations. In contrast, uridine, deoxyguanosine, glutamine, choline chloride, triethanolamine, glycylglycine, and sodium acetate, all of which were incorporated into their medium, were not stimulatory to the type b strain. These latter substances, with the exception of sodium acetate, were also omitted in the medium described by Butler. Counter to our experience, Butler did not find arginine to be an absolute requirement, nor was biotin or inositol stimulatory to growth. It would therefore appear from the results obtained in these recent investigations that different strains or types of *H. influenzae* may vary in their nutritional requirements.