PRO EXPERIMENTIS

Growth of Hemolytic Streptococci in Vitamin Free Medium[1]

Streptococcus pyogenes bypasses its eight special nutritional essentials by conversion to the L form in vitamin free medium. This paradoxical growth has several interesting aspects. By analogy, one can speculate that organisms with nutritional complexities almost precluding their culture may be expected to grow rapidly in the L cycle. In this respect it is pertinent that *Mycobacterium tuberculosis* develops in 24 h in the L form[2]. Admittedly, specific identification of such growth may be difficult. However, if the organism is anticipated, identification may be made with appropriate antiserum, perhaps with fluorescent labeling.

Culture of fastidious bacteria on neutralite free medium also increases the scope of bacterial physiology. Growth requirements must be determined for both the classical stage and its L form. Fortunately, requirements for L form development are similar for widely different genera. Thus, a medium originally devised for the L form of *Proteus* sp.[3] will also support the L form of other *Enterobacteriaceae*[4], of *Staphylococcus aureus*[5], *Mycobacterium* sp.[2], and *Candida* sp.[6]. This defined medium is the subject of this paper.

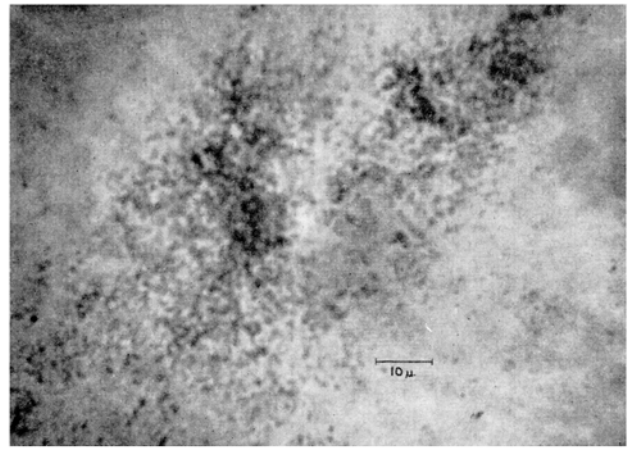
The medium is a modification of one described by MEDILL and O'KANE[3]. It consists of 0.1% dextrose, 10.0% sucrose, 0.8% Noble's agar (Difco), 2.0% sodium lactate, 0.9% K_2HPO_4, 0.2% salts, 0.5% vitamin free casamino acids. The salt mixture contains: $MgSO_4 \cdot 7H_2O$ 40 g; NaCl 2 g; $FeSO_4 \cdot 7H_2O$ 2 g; $MnSO_4 \cdot 4H_2O$ 8 g. The salts were homogenized by trituration. Double strength agar was autoclaved separately. All other ingredients were mixed, put in solution in double strength and filter sterilized.

This defined medium can be obtained commercially, packed as a sterile solution which requires only the addition of freshly autoclaved double strength agar and sucrose.

Streptococci grew in the L form in the defined medium without bacteriostatic agents. The ability to form L colonies in this medium appeared characteristic of streptococci in general, since none of the seven strains were refractory. The L form seems as natural as the classical stage and will develop even with simple nutrition, if toxic agar and other ingredients which inhibit the L form are avoided.

Six of the streptococcal strains belonged to Group A; one was *Streptococcus faecalis*. To prepare the inoculum organisms from an 18 h culture in tryptose phosphate broth were washed three times and diluted in saline before standardized numbers were cut into the surface of the soft agar plates.

The morphology of the L growth is shown in the accompanying micrograph. The elements of a colony break apart with slight disturbance, necessitating that photographs of colonies be made *in situ*. The areas which are in focus show the multiple branching which was similar for all the strains of streptococci. Repeated examinations of colonies in thin preparations revealed no classical streptococcal forms. The ease with which segments of L growth separate suggests that the substances responsible for coccal alignment in streptococci and the cell wall itself are deficient in the variant. The colonial growth was consistently about 0.5 mm subsurface despite anaerobic incubation of the cultures. Thus it appears that lowered oxygen tension is critical. Microscopic L forms were present after 24 h incubation at 37°C; macroscopic cloudiness along the line of streak was visible after further incubation.



Filamentous branching growth of the L stage of β-hemolytic Streptococci

This micrograph is remarkably similar to one of streptococcal L forms by PANOS and BARKULIS[7] except for absence of large bodies which typically result from high concentrations of penicillin. Previous studies on this genus have usually employed penicillin in a concentration of 1000 units/ml or more, following the original observation of DIENES and SHARP[8].

Reversion of the L filaments to cocci occurred in two of the Group A and in the Group D streptococcal strains 24 h after subculture to tryptose agar enriched with sheep blood. No reversion occurred for the other four strains.

The concept that L formation is normal does not contradict the evidence that L forms can be artificially stimulated in media usually devoid of them, when penicillin interrupts cell wall synthesis. It has been shown for Group A streptococci, for instance that the stage induced by penicillin lacks rhamnose and hexosamine[9], constituting an explanation for the lack of rigidity of the wall.

The medium described in this paper has also proved useful for recovering streptococci *in vivo*. A patient with subacute bacterial endocarditis did not respond to heavy dosage of penicillin. Meningitis developed as a complication, with a spinal fluid cell count of 4000/cm³. Repeated cultures of blood and of spinal fluid resulted in no growth. The defined medium was tested, with the agar content lowered to 0.1%. Horse serum (2.0%) was added to enrich

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the spinal fluid culture. In 24 h both cultures contained numerous spheroplasts, a few streptococci and highly pleomorphic cocci. When transferred to blood agar the colonies were predominately tiny and the inhibition zones of antibiotic sensitivity tests were read with a dissecting microscope. The organisms from blood and spinal fluid appeared identical, with only a few typical α-hemolytic colonies on first subculture, but with growth becoming entirely typical after three subcultures. After reversion the organisms exhibited the same growth characteristics as the streptococci from cultures before therapy was instituted.

With present knowledge it cannot be stated whether the streptococcus existed in the patient in the L stage, or whether growth was initiated in the L stage because only a few organisms were in the specimens. It has been shown with other cocci that when the inoculum is minimal growth may be of the L type[5].

VIGOUROUX and HANNOUN[10] have found L forms in blood cultures from streptococcal endocarditis in several instances. They have, furthermore, shown that the streptococcus can assume the L form *in vivo* and has an affinity for red corpuscles. In their opinion, MULÈ's[11] intraerythrocytic bodies of scarlet fever and rheumatic fever probably represent streptococcal L forms. The association of streptococcal L forms with erythrocytes was also observed in our cultures.

Zusammenfassung. Es wird gezeigt, dass die L-Form verschiedener Streptokokken auf einfachem vitaminfreiem Nährboden wachsen kann, dessen genaue Zusammensetzung und Herstellung beschrieben wird. Auf das morphologische Wachstumsverhalten der Streptokokkenkolonnen bei Benutzung der synthetischen Nährböden wird ausführlich eingegangen.

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DISPUTANDUM

De l'opportunité de quelques équations différentielles en vestibulométrie

C'est à juste titre que le labyrinthe a été ainsi dénommé par les anatomistes d'autrefois. Les physiologistes après eux en ont cherché le fil d'Ariane: ils s'accordent tous à reconnaître les difficultés auxquelles ils se heurtent au cours d'une telle recherche. Aussi, est-ce avec une certaine stupéfaction qu'on a vu les physiciens se mêler d'un problème déjà si complexe et dont les éléments expérimentaux reproductibles sont si peu nombreux; cette carence de données valables explique sans doute la propension que les disciples de DESCARTES ont manifesté à l'égard de l'analogie.

C'est en effet sous la forme d'une séduisante analogie physico-mathématique qu'un théoricien hollandais[1] a présenté le fonctionnement de la cupule labyrinthique, il y a quelques années. Cette analogie n'avait en elle-même rien de répréhensible – car chacun peut comparer la cupule à un pendule de torsion et relever dans un traité de physique classique les fondements mathématiques justificatifs de sa comparaison. Néanmoins, à la lumière des travaux[2–4] qui permettent de voir *in vivo* le «déplacement» cupulaire au cours des différents mouvements de l'animal, on hésite d'emblée à considérer la cupule comme un *pendule* répondant à l'équation:

$$\theta\xi' + \pi\xi'' + \Delta\xi''' = 0$$

(où θ représente le moment d'inertie de la cupule, où π représente le moment de friction/unité de vit. angul., où Δ représente le moment directionnel/unité d'angle, où les trois facteurs ξ représentent respectivement la déviation, la vitesse et l'accélération angulaires de l'endolymphe relativement à celles du crâne).

Il y a à cela deux raisons: la première est qu'il est actuellement impossible de mesurer chez l'homme *d'une manière directement reproductible* les trois constantes et les trois facteurs ci-dessus. Cette raison suffirait à elle seule à rayer définitivement du monde des ingénieuses constructions une telle équation différentielle;

la seconde raison est fournie par le fait que les données les plus classiques[5,6] aussi bien que les plus récentes[7] d'histo-physiologie sensorielle s'accordent à mettre en évidence des phénomènes non linéaires ou mieux, non linéairement amortis dans le temps; l'un des arguments les plus péremptoires réside dans le fait que l'anisotropie de la cupule lui confère des propriétés mécaniques différentes selon les directions.

L'équation de GROEN se trouve ainsi réduite à demeurer à la rigueur une *équation d'état*, sans applicabilité, par conséquent, pour le chercheur ou pour le clinicien, l'un et l'autre devant admettre a priori ce postulat restreint.

Les labyrinthologistes hollandais pourtant, sous l'impulsion de l'école d'Utrecht, ont décidé d'ériger le postulat en loi quasi-générale, l'hypothèse de travail en preuve: le fruit de cette transformation a reçu le nom de cupulométrie[8,9]. Il apparaît pourtant à un esprit scientifique rigoureux qu'un tel sophisme ne devrait pas être possible à notre époque; il semble surtout qu'un tel artifice ne devrait pas permettre la mise en doute des principes physiologiques les plus appuyés par une longue expérience, telle les lois d'EWALD et de BARANY (connue sous le nom de 2e loi d'EWALD)[10,11], la valeur du seuil nystagmique

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