FOLIA MICROBIOLOGICA

Vol. 5, 1960

A Reverse CAMP Diagnostic Test with *Corynebacterium pyogenes* varietas *hominis*

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Received July 28, 1959

Corynebacterium pyogenes varietas *hominis* is a Gram-positive rod described by Patočka (1955) and others, which in recent years has often been found to be the cause of different suppurating processes in man, including osteomyelitis, suppurating discharges and tonsillitis.

Patočka and Schindler (1956) also described a series of diagnostic signs for the differentiation of human and animal Corynebacteria. Since colonies of *Corynebacterium pyogenes* var. *hominis* on sheep blood agar often resemble colonies of haemolytic beta streptococci, they frequently escape notice.

Group B beta haemolytic streptococci can be diagnosed by means of the CAMP test (Christie, Atkins & Munch-Peterson, 1944). This test is based on the finding that alpha-beta staphylococcal haemolysis is intensified if crossed by group B streptococci (Fig. 1).

It was found that *Corynebacterium pyogenes* var. *hominis* had precisely the opposite effect on staphylococcal haemolysis on blood agar containing sheep erythrocytes, since at the site at which the staphylococcal and *Corynebacterium pyogenes* inoculation lines crossed, triangular inhibition of staphylococcal haemolysis occurred, showing that these Corynebacteria release a substance with an inhibitory effect (Fig. 2, 3). As seen from these figures, *Corynebacterium pyogenes* var. *hominis* forms at least two easily distinguishable substances, which are diffused into the parts surrounding the colony:

(a) one of these forms a narrow line of beta haemolysis below the colony, in the immediate vicinity of the inoculation line, if sheep, rabbit and human erythrocytes are used;

(b) the other substance is diffused particularly clearly in sheep blood agar for a considerable distance, and produces plainly discernible inhibition of staphylococcal beta haemolysis.

A narrow line of intensified haemolysis develops on the dividing line between the areas of inhibition and staphylococcal haemolysis.

Substances effective against staphylococcal haemolysin can also be demonstrated by saturating strips of sterile filter paper with a three- to nine-day broth culture containing 10—20% horse serum, allowing the excess fluid to drip off, placing the strips on an agar plate containing sheep blood and leaving it for five minutes in a thermostatic incubator at 37° C so as to permit diffusion of the filtrate into the agar medium. The strips of paper are then removed and the medium is inoculated with a staphylococcal culture at right angles to the direction of the strips. Staphylococcal haemolysis is likewise inhibited in this case.

Inhibition of staphylococcal haemolysis was demonstrated *in vitro* by using filtrates from a broth culture of *Corynebacterium pyogenes* var. *hominis*. The experiment was carried out as follows:

A broth culture of Corynebacteria, freed from the actual bacteria, was pipetted in progressive double dilutions into Kahn test-tubes up to a dilution of

1 : 32, in amounts of 0.5 ml. and the same amount of 1% rabbit erythrocytes was then added. The mixture was left for 30 minutes at room temperature, after which 0.5 ml. staphylolysin was added. The original titre of the staphylolysin, which was diluted 160-fold, was 1 : 160.

The test-tubes were then incubated in a water bath at 37° C for 30 minutes and after a further two hours the degree of inhibition of haemolysis was read off at laboratory temperature. Inhibition was usually determined under these conditions in the first or second test-tube only. Since the weak haemolysins in the Corynebacterium filtrate had an adverse effect on inhibition of staphylococcal haemolysis, the experiment was modified by heating the culture fluid for 20 minutes at 56° C, thus inactivating the Corynebacterium haemolysins (Tab. 1). As a result, staphylococcal haemolysis was still inhibited in the fourth test-tube (dilution 1 : 8).

Table 1. Inhibition of staphylococcal haemolysins by fluid decanted from culture of *Corynebacterium pyogenes* var. *hominis*

Dilution of fluid	Inhibition of one unit staphylolysin
1 : 1	+
1 : 2	+
1 : 4	+
1 : 8	±
1 : 16	—

If Corynebacterium filtrate was mixed with staphylolysin before adding the erythrocytes, inhibition of haemolysis was much weaker.

It was found that animal strains of *Corynebacterium pyogenes*, *Corynebacterium diphtheriae* (all types), *Corynebacterium pseudodiphtheriae* and other bacteria did not possess this property (Tab. 2).

Staphylococci affecting other staphylococci in the same way have also been described (Liu, 1954; Flamm, 1957) and have been observed by the present authors.

Table 2. Inhibition of staphylococcal haemolysins by different species of bacteria on blood agar containing sheep blood during 24 hours

Strain	No. of strains	Inhibition of haemolysis
Corynebacterium pyogenes var. *hom.*	19	+
Corynebectarium pyogenes bovis ÚEM, Praha	2	—
Corynebacterium pyogenes bovis SVVÚ, Praha	9	—
Corynebacterium pyogenes animale, Budapest	3	—
Corynebacterium equi SVVÚ, Praha	1	—
Corynebacterium diphtheriae typ. gravis	5	—
Corynebacterium diphteriae typ. intermedius	2	—
Corynebacterium diphtheriae typ. mitis	200	—
Staphylococcus pyogenes	200	—
Staphylococcus pyogenes	5	+

The above experiments were carried out with 19 strains of *Corynebacterium pyogenes* isolated from man, using 15 animal strains, five strains of *Corynebacterium diphtheriae* typus gravis, two of typus intermedius and 200 of typus mitis as controls. They supplement, or rather rectify, the findings of Fried (1956), who erroneously ascribed the above property of *Corynebacterium pyogenes* var. *hominis* to *Corynebacterium diphtheriae* typus mitis.

In the authors' opinion—which has been confirmed by their own experiences—this test greatly simplifies the diagnosis of human strains of *Corynebacterium pyogenes* and will permit much more frequent demonstration of *Corynebacterium pyogenes* var. *hominis* in human pathological material.

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ОБРАТНЫЙ CAMP-ТЕСТ КАК СРЕДСТВО ДЛЯ ДИАГНОСТИКИ CORYNEBACTERIUM PYOGENES VARIETAS HOMINIS

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Штаммы Corynebacterium pyogenes varietas hominis, изолированные из различного патологического материала от больных людей, подавляют стафилококковый гемолиз на кровяном агаре с бараньей кровью особенно интенсивно.

Таким образом, этот обратный CAMP-тест характерен для всех выделенных от человека штаммов этого микроба и имеет диагностическое значение при дифференциации от штаммов Corynebacterium pyogenes, Corynebacterium diphteriae typus gravis, mitis и intermedius, выделенных от животных.

Угнетающее действие было количественно определено in vitro до титра максимум 1 : 8. Описываемый обратный CAMP-тест является важным диагностическим средством для идентификации этого мало изученного микроба.

Manometric Evaluation of the Efficiency of Phosphate Fertilizers according to their Effect on Soil Microflora

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Received June 24, 1959

Warburg's respirometric method was used to determine the biological efficiency of phosphate fertilizers, the method being of late frequently applied to the investigation of processes taking place in the soil (Drobník, 1957). It is known from the work of Harden and Young (1905) that the rate of decomposition of saccharides is significantly increased in the presence of inorganic phosphates. Chang (1940) and Kaila (1949) showed that, during incubation of a soil with sugar and with a soluble

Table 1. Characteristic of soils used in the experiments

	pH		$CaCO_3$	Acidity according to Kappen (y_1)	% humus
	H_2O	KCl			
Calciferous alluvial soil from the Danube (F)	8.5	8.2	28.14	—	3.05
Acid forest soil (B)	5.3	4.6	—	19.92	2.80