CONTRIBUTION TO THE STUDY OF THE CRYSTALS FORMATION PROCESS BY BACTERIA

by

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Many workers have pointed out the formation of calcium carbonate crystals by bacteria; McCallum, M. F. and Guhathakurta, K. J. (1.970) reported the precipitation of calcium carbonate in the form of aragonite in sea water and calcite in fresh water.

More recently Boquet, E., Boronat, A., and Ramos Cormenzana, A. (1.973) have shown that all bacteria tested on a medium described by them, produced crystals. Thet suggested that calcite formation is a universal process and although Shinano, J. (1.972) published data which indicated that Gram positive spore-forming bacteria are incapable of producing calcite crystals, Ramos-Cormenzana, A. (1.975) proved this formation by bacteria of the genus *Bacillus.*

Many others workers, Ruellan, A. (1.971) have suggested that, by nature, this process may lead to the creation of a calcic horizon.

This study was designed to analize the process of crystals growth formed by bacteria in different conditions, so as to know the type of crystals formed.

MATERIAL AND METHODS .-

ORGANISMS

The cultures used in this study were : *Escherichia coli*, *Bacillus sphaericus*, *Bacillus brevis*, *Micrococcus luteus* and a pathogenic strain, *Salmonella typhimurium.*

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MEDIA

Two media have been selected for this purpose. The BRC and the B-19 as previous reported by Ramos-Cormenzana, A.; Perez-Miranda, C, and Boquet, E. (1.975).

TECHNIQUES FOR INOCULATION OF MEDIA

Two methods have been used. The streak method and the drop method (placing only one drop of bacterial suspension in the middle of the plate). All plates were incubated at 25º C and one experiment was done at 37ºC.

ANALYSIS OF CRYSTALS GROWTH

We used an image tv analyzator equipment Microvideomat Zeiss with an incorporated photomicroscope.

IDENTIFICATION OF CRYSTALS FORMED

Crystals were harvested from colonies as previous repport by Ramos-Cormenzana, A. (1.975). The identity was made by x-ray diffraction and confirmed by chemical analysis using atomic absorption.

RESULTS AND DISCUSSION.-

1.- MORPHOLOGY OF CRYSTALS

A.- INOCULATION IN MEDIUM BRC

a).- Streak inoculation.

In these conditions we can observe that the crystal growth may be produced either as isolated crystals or as grouped crystals within the colony as may be appreciate in the photograph 1.

In the isolated crystals it may be recognized the ro sette growth, proper of the aragonite crystals as Boquet et al. (1.973) have pointed out. (phot. 1).

A fact subject of discussion was if the crystal growth in solid media is a factor depending of the own bacteria, or on the contrary, if it is a mechanic effect. In phot. 2 it may be observed the influence of bacteria (Salmonella typhimurium as in the former cases) because we can observe that where there is less bacterial mass, there is not crystal growth, while there are crystals in the part where there's more bacterial growth.

Photographs 3 and 4 are also very demostrative of this fact because we can see how the crystal growth is larger inside the colony. In these photographs it can also be observed the different density of crystals within each colony.

With reference to the distribution of the crystal growth may be observed in the photograph 4 that there are central voids at the different colonies which are destituted of crystals while the edges of the colonies are completely full. So the growth has a peripheric distribution which suggestes the origin of the pedodes formation.

b) .- Drop inoculation

This method of inoculation was used, to eliminate the mechanical efect. We can see in phot. 5 how the crystals formation have taken place and besides they have a different morphology with regard to those of the former medium (instead of it, is the same bacteria inoculated in the same medium). The crystals are euhedric, they have geometrical shapes and we can observe that the distribution of the growth is very different to the former one because the growth have placed in the middle of the colony and not in the edges.

B.- INOCULATION IM MEDIUM B-19.

In this medium we can observe how the morphology of the crystals is quite different to those of the medium BRC and besides, there are differences according to the used bacteria. The crystals formed by Escherichia coli present a different morphology to those of the medium BRC and as it ocurred formerly, they have the center with less crystalinity. The same bacterium inoculated at 37ºC forms crystals a little different to those we have seen till now (phot. 6).

The crystals formed by Micrococcus luteus present a similar morphology to those formed by Escherichia coli inoculated by drop method and also present the center, with less crystalinity (phot. 7).

The crystals formed by Bacillus brevis are similar to those formed by E. coli inoculated by streak method.

In phot. 8 we can observe the crystals formed by B. sphaericus and we can see how the growth have taken place in and outside the microbienne mass and shows a tendence to be situated in the middle of the colony.

In the observations of the graphics of crystal growth (figures 4 and 5) we will see how in some cases there exists a decrease in the amounts of crystals formed by several bacteria, we think it may be due to a redisolution of the crystals, becoming gels as we can see in phot. 9.

This occurs both at the drop inoculation and at the streak inoculation methods. In the case of crystals formed by E. coli we were able to observe the growing on process (as it may be seen in phot. 10) during many days, without any contamination how it had placed the formation of a sort of crust.

2.- NATURE OF THE CRYSTALS FORMED.-

A.- BRC MEDIUM.

The x-ray diagram common to 8 samples analyzed is as follows :

d (Å)	Intensities
3,41	10
2,92	1
2,77	1
2,39	6
2,11	1
1,99	4
1,93	0,5
1,86	2
1,77	4

The chemical composition of these crystals is as follows :

CO_2 = 42,80 % CaO = 50,72 %

SrO = 0,18 % MgO = 6,10 %

In the sight of such results, we plainly observe that it is about aragonite with a high proportion of magnesium wich makes to turn aside a little its own reflections.

B.- B-19 MEDIUM.

The x-ray diagram common to all the formed, crystals by the different used bacteria is as follows:

d (Å)	Intensities
4,30	0,5
3,48	10
3,02	2
2,82	1
2,45	5
2,16	0,5
2,04	3
1,98	0,5
1,89	3

The chemical composition of these crystals is as fo llows :

CO_2 = 30,84 % CaO = 10,05 %

SrO = 60,01 % MgO = 0,22 %

In the light of these results, we can clearly obser ve that it deals with a calcio-stroncianite.

3. - MEASURE OF THE GROWTH .-

The figures have been established with the media of the measures brought about at the Petri dishes used, generally 6, though in some case we had to reject some Petri dish for being contaminated.

In a general way we can see that the growth is si milar in any medium and the different bacteria, although there are some differences.

The growth at the streak inoculation (fig. 6-11) has 100 more times than at the drop inoculation (fig. 1-5) - that is the reason why we use a different scale for the re presentation of growth.

In most cases we can observe a great rate of growth which begins about the day 10th. It was lowered in other, cases as in B. brevis, in which the fact took place the day 20th.

The decrease of the crystals formation that we can observe in some cases at the end of certain time, is variable according to the different bacteria (figures 4, 5). This fact, as we have said formerly, appears to be due to a redisolution of crystals transforming itself in gels conserving a cristalline habit.

Fot. 1.- Salmonella typhimurium. BRC medium. Streak inoculation. 10 x (only polarizator).

Fot. 2.- Salmonella typhimurium. BRC medium. Streak inoculation. 40 x (only polarizator).

Fot. 3.- Salmonella typhimurium. BRC medium. Streak inoculation. 10 x (only polarizator).

Fot. 4.- Salmonella typhimurium. BRC medium. Streak inoculation. 10 x (crossed polarizator).

Fot. 5.- Salmonella typhimurium. BRC medium. Drop inoculation. 40 x (only polarizator).

Fot. 6.- Escherichia coli. B-19 medium. Streak inoculation. 10 x (oblique nicols).

Fot. 7.- Micrococcus luteus. B-19 medium. Streak inoculation. 40 x (crossed polarizators).

Fot. 8.- Bacillus shaericus. B-19 medium. Streak inoculation. 10 x (oblique nicols).

Fot. 9.- Bacillus brevis. B-19 medium. Streak inoculation 40 x (oblique nicols).

Fot. 10.- Escherichia coli. B-19 medium. Drop inoculation. 10 x (only polarizator).

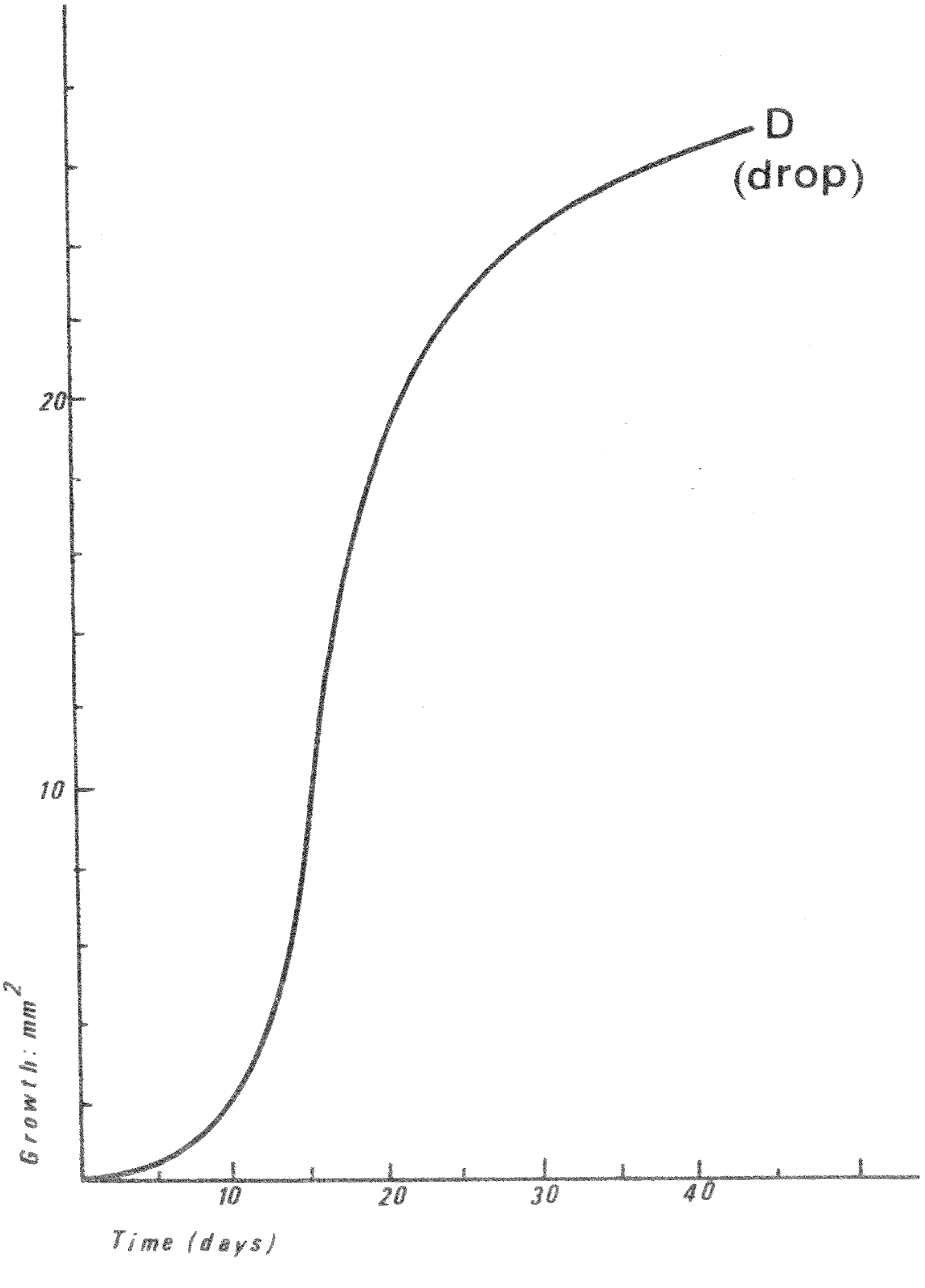


Fig.1 S.typhimurium M:BRC

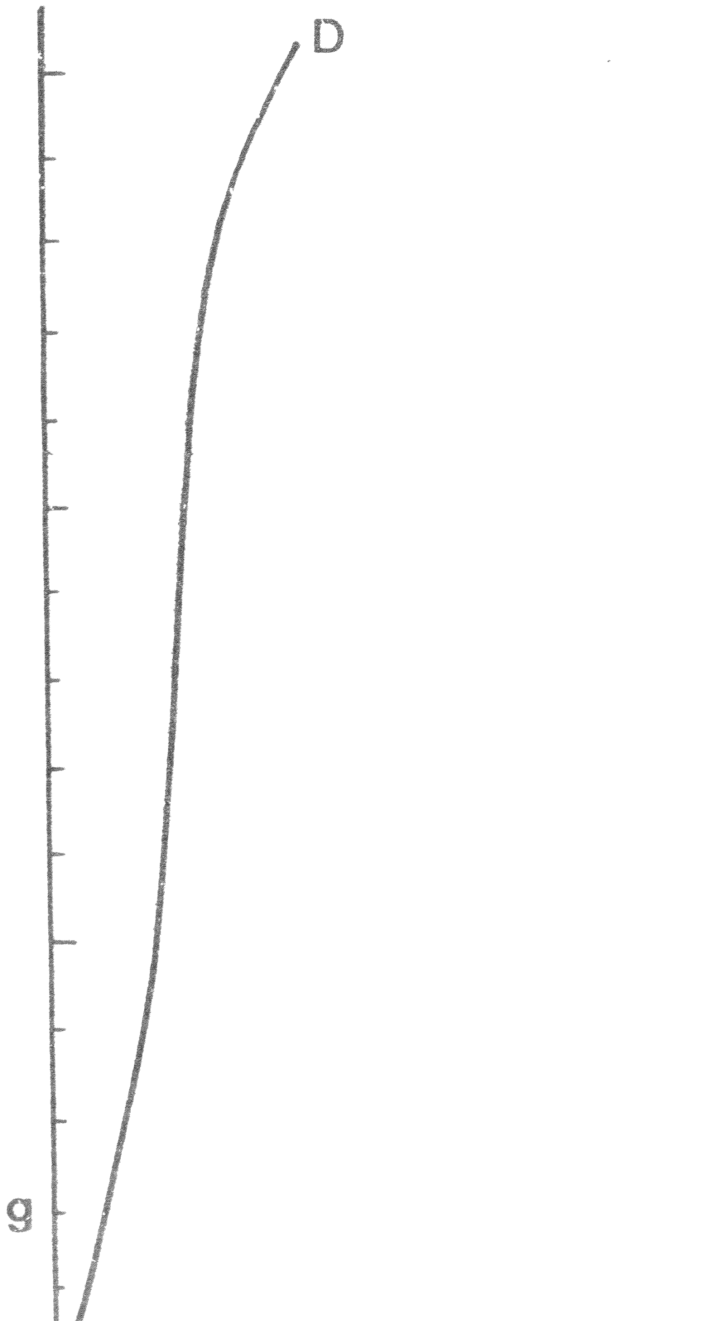


Fig.2 E.coli M:B 19

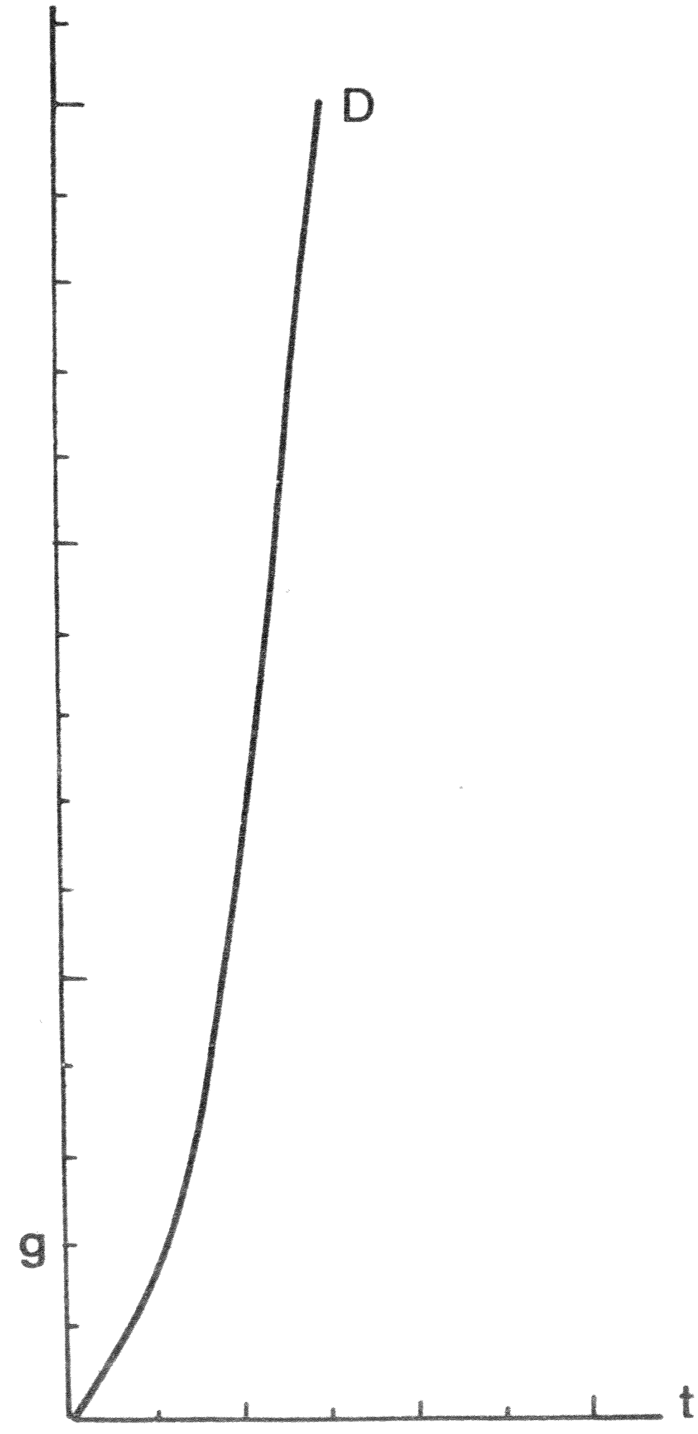


Fig.3 M. luteus M:B 19

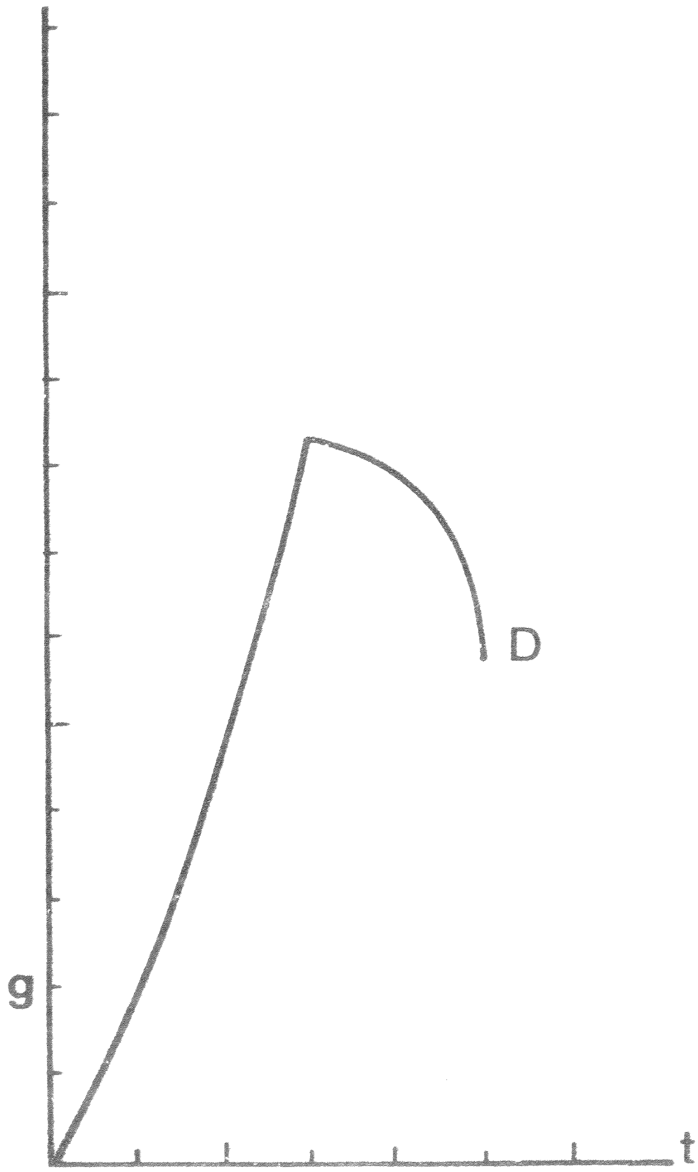


Fig.4 E.coli M:B 19

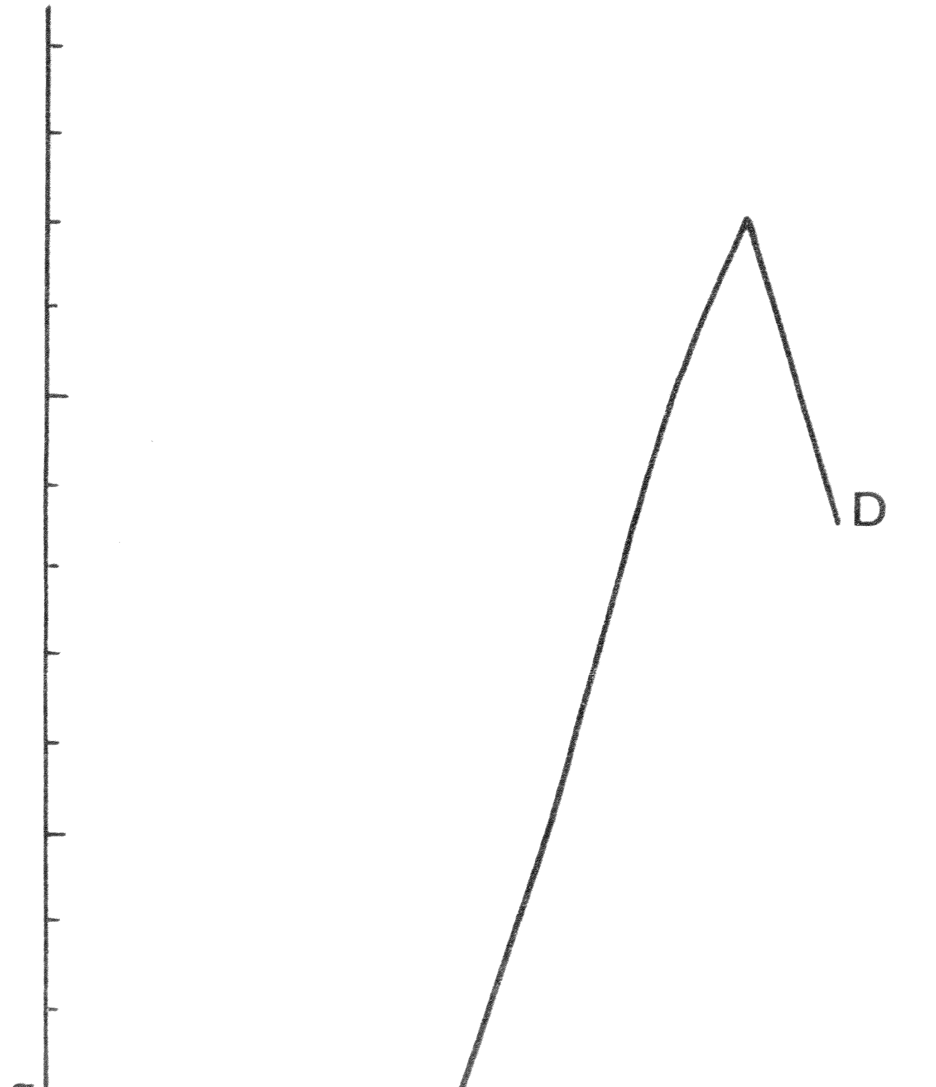


Fig.5 E.coli M:B 19

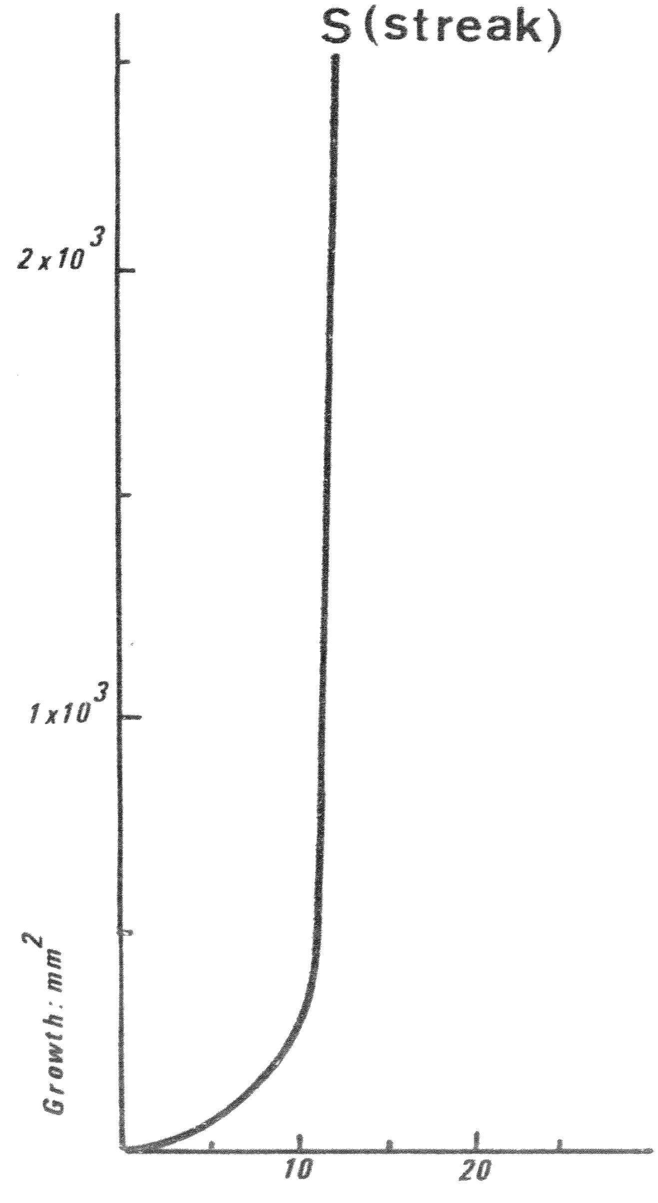


Fig.6 S.typhimurium M:BRC

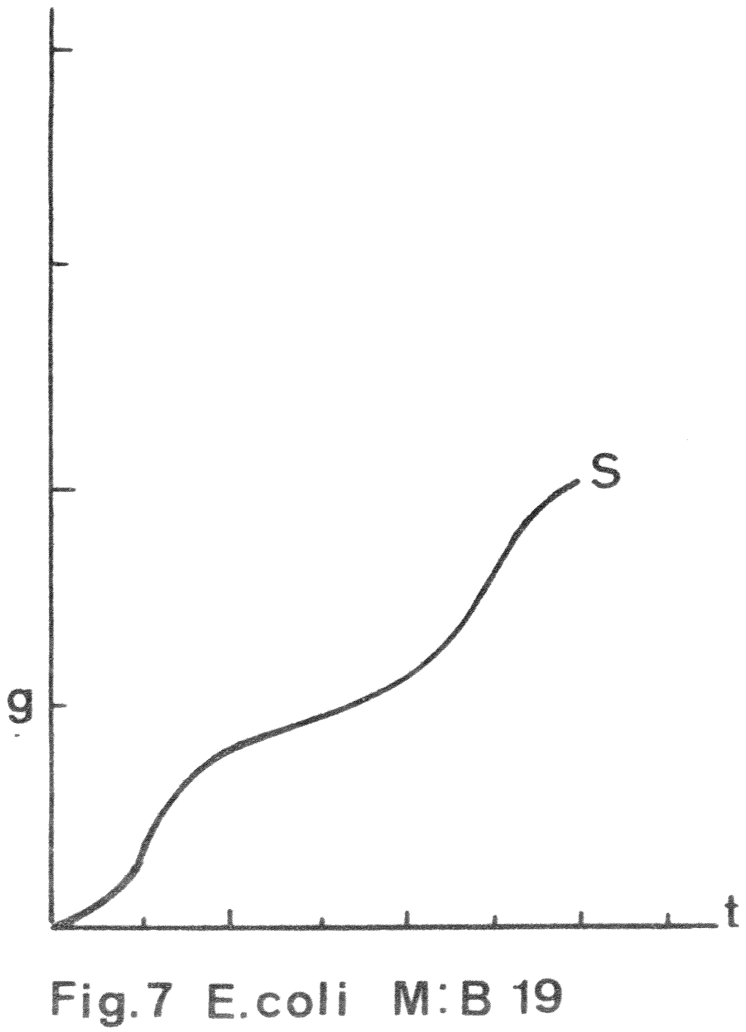


Fig.7 E.coli M:B 19

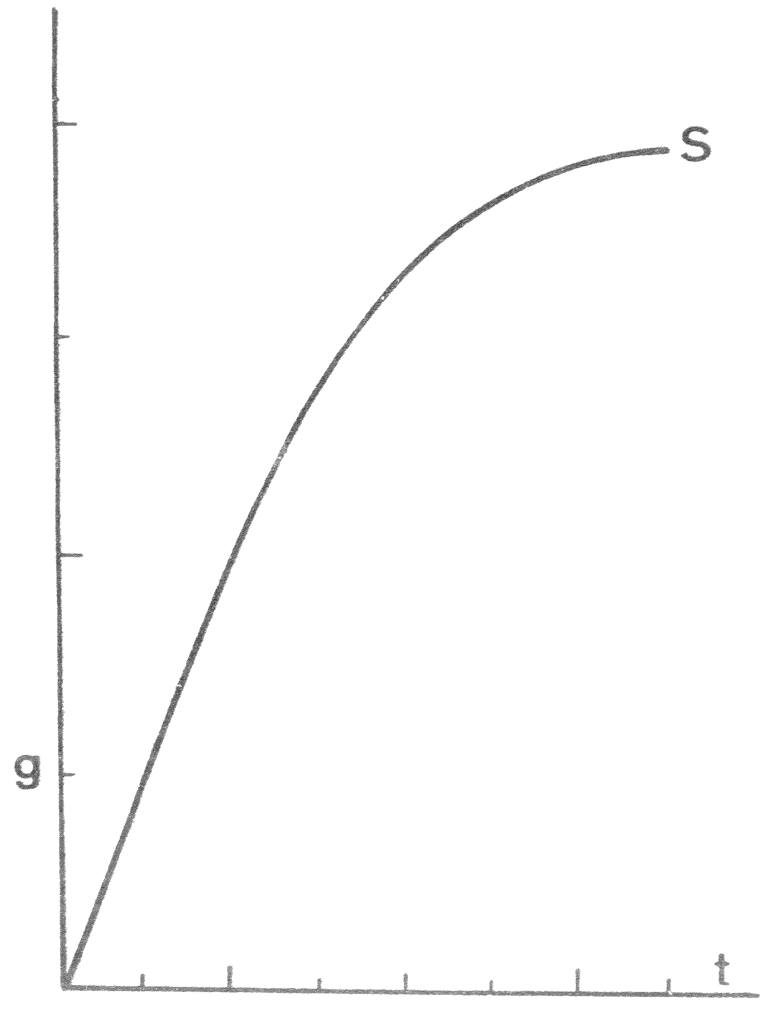


Fig.8 M.luteus M:B 19

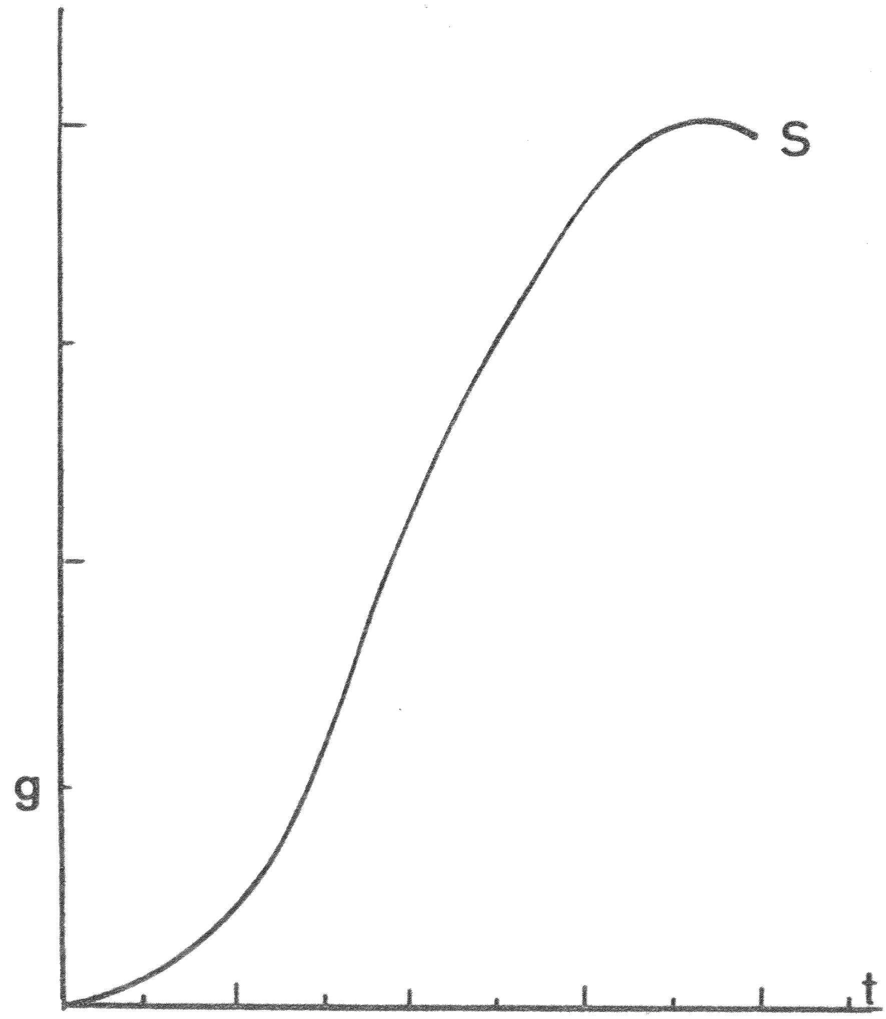


Fig.9 E.coli M: B 19

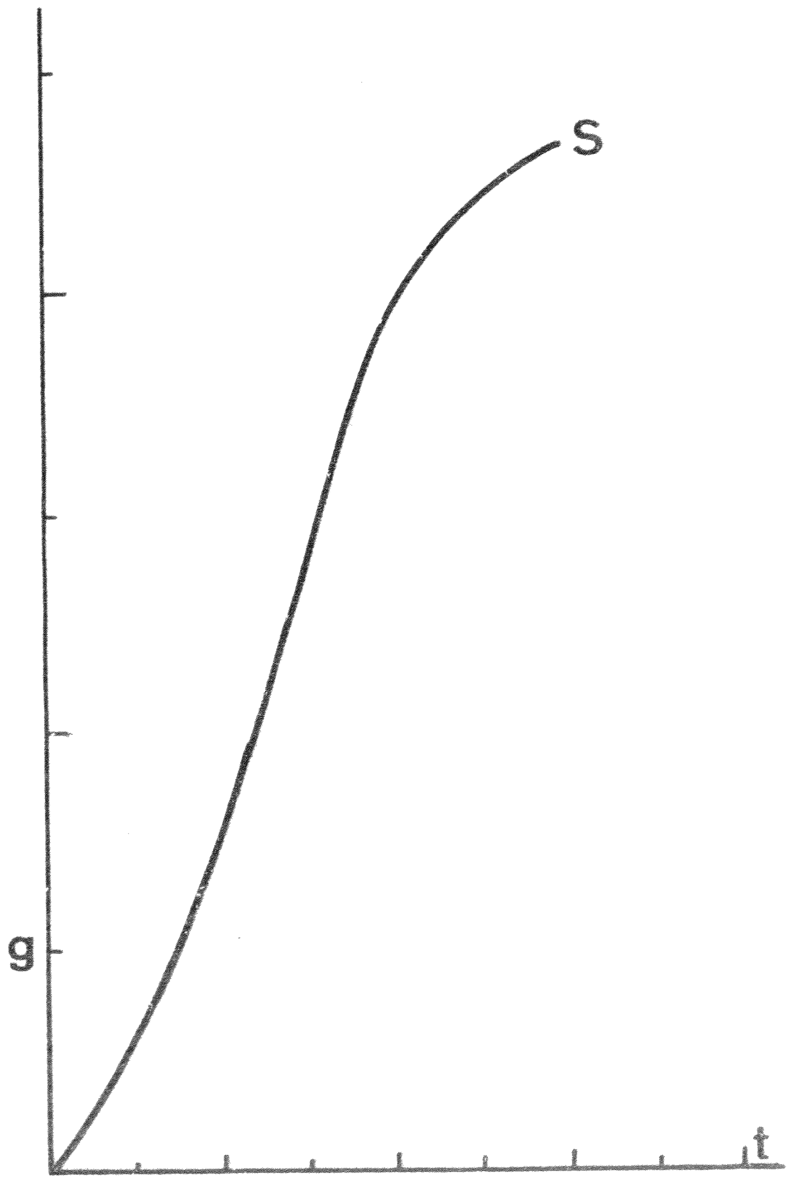


Fig.10 B. sphaericus M:B 19

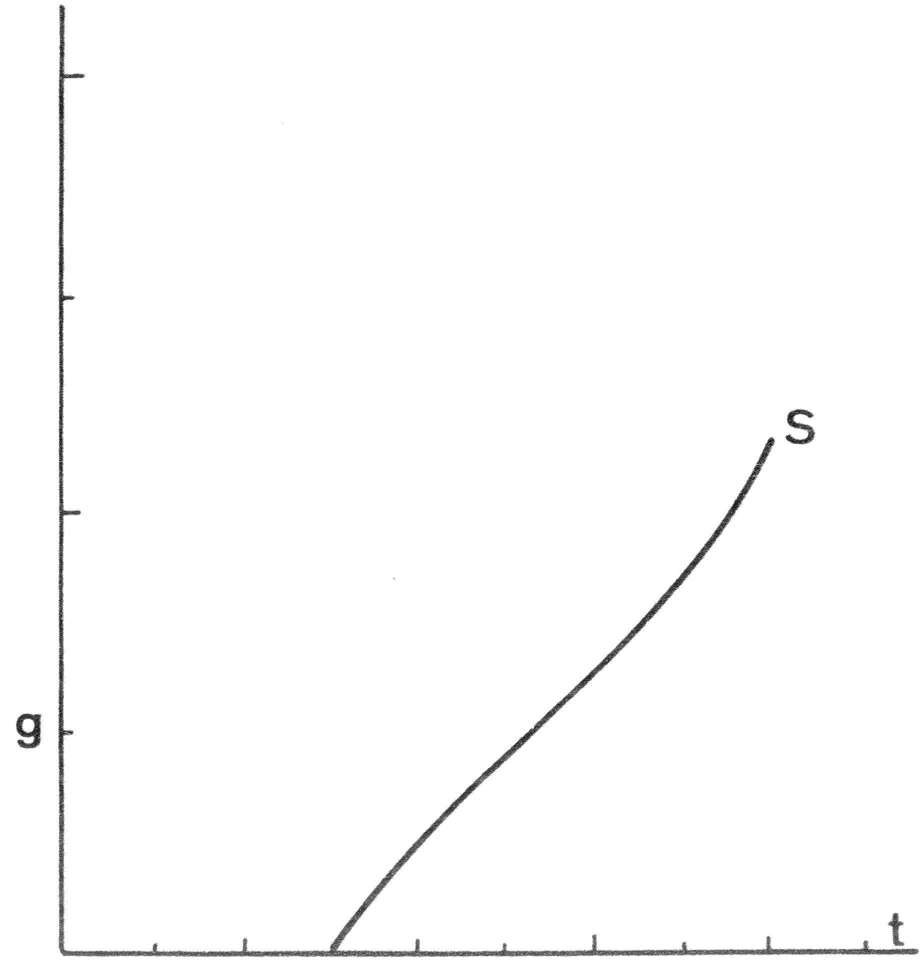


Fig.11 B.brevis M:B 19

CRYSTALS FORMATION BY BACTERIA

SUMMARY

It has been studied the morphology and distribution of the crystals formed in several solid media by different bacteria (E. coli, Bacillus brevis, B. sphaericus, Micrococcus luteus and Salmonella typhimurium). We gibe a possible explanation of the origin of pedodes and calcareous crusts. Samely it has been studied the nature of the crystals formed by X-ray and chemical analysis and it has been established the graphics of crystals growth by different bacteria along the time.

REFERENCES.

Boquet, E, Boronat, A., and Ramos-Cormenzana, A. (1.973). Nature. 246, 527-529.

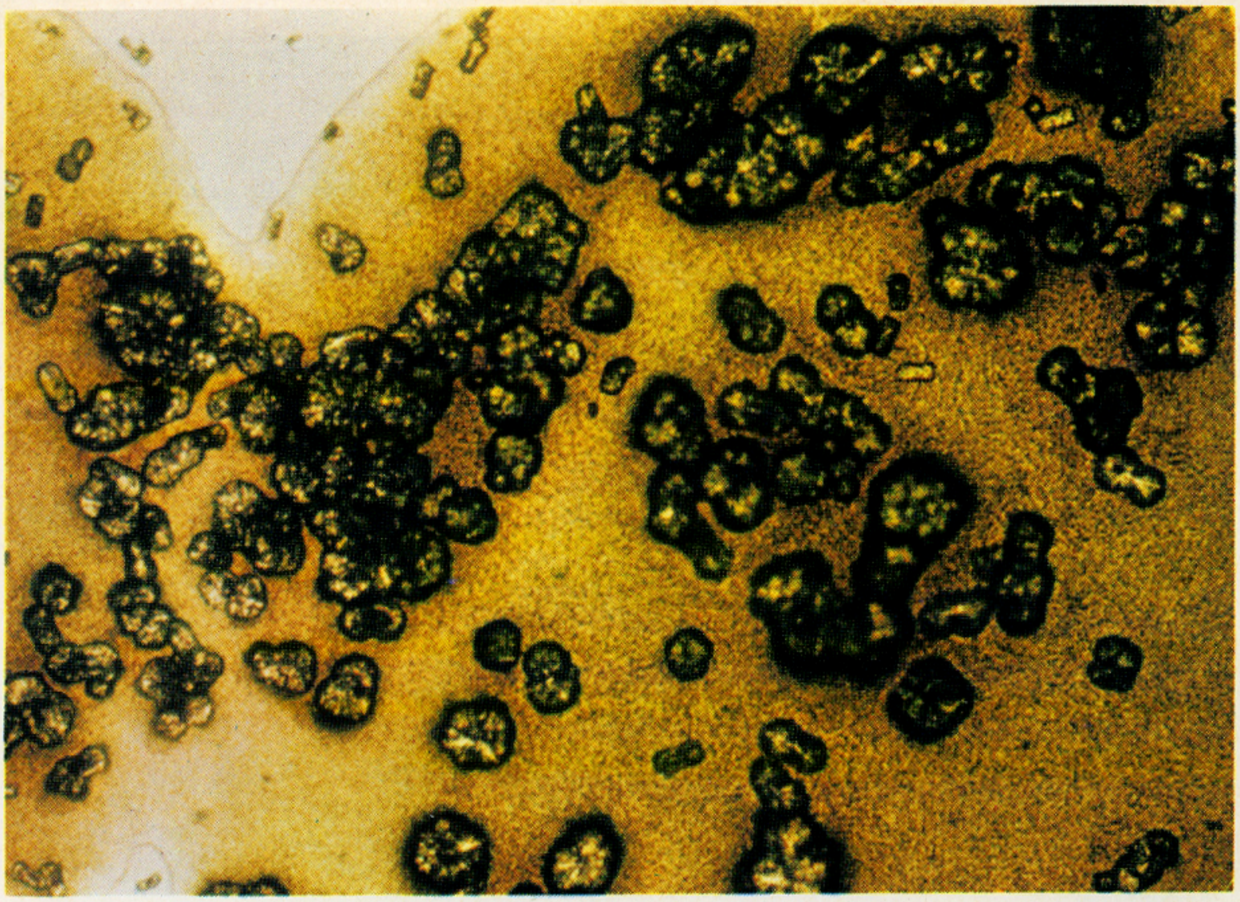
McCallum, M., and Guhathakurta, K. (1.970), J. Appl. Bact. 33, 649-656.

Ramos-Cormenzana, A. (1.975), Microbios, 13, 61-70.

Ramos-Cormenzana, A., Perez-Miranda, M.C., and Boquet, E. (1.975). Ars Pharm. 26, 335-343.

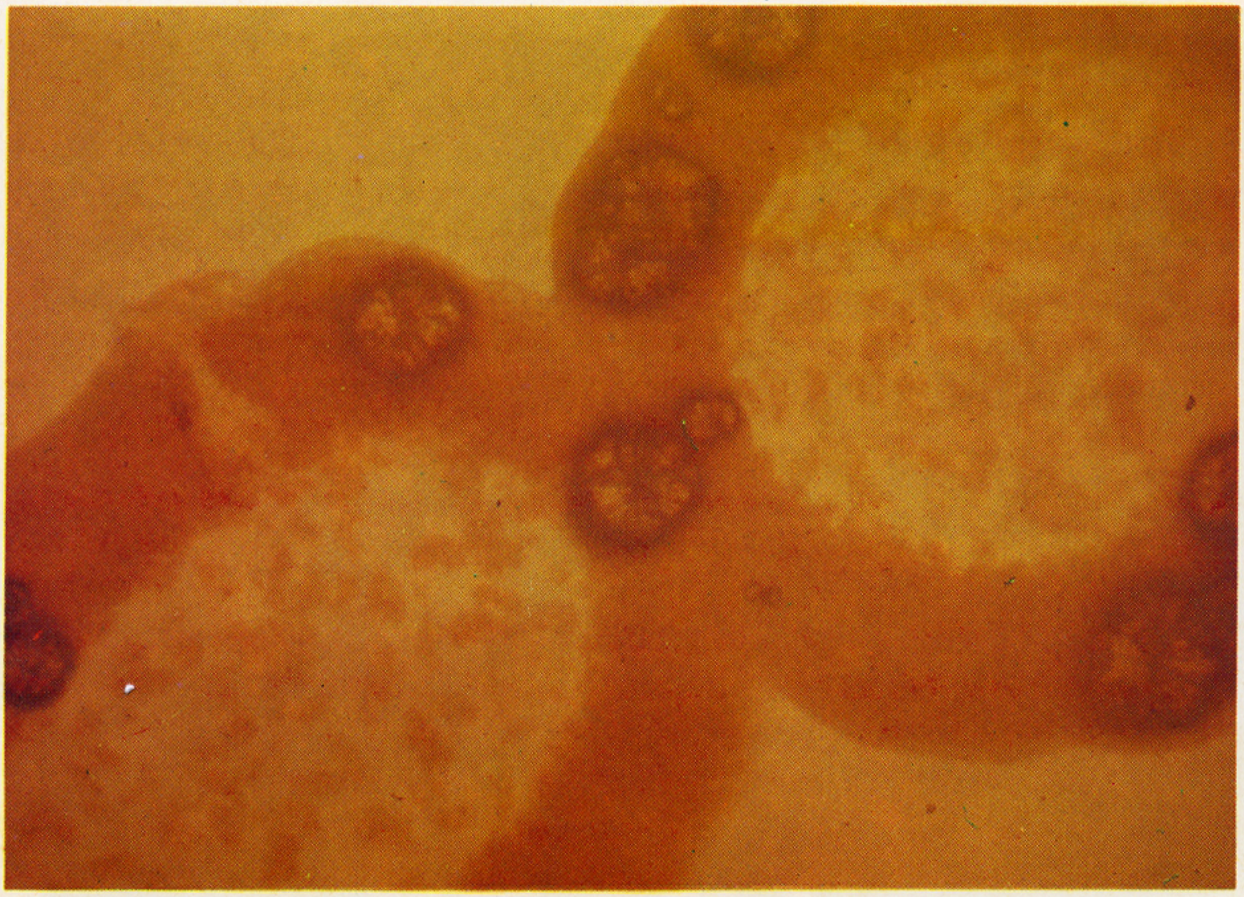
Ruellan, A. (1.971). Mémoires orstom nº 54. 302. Paris.

Shinano, J. (1.972). Bull Jap. Soc Sci Fish, 38, 717 - 723.

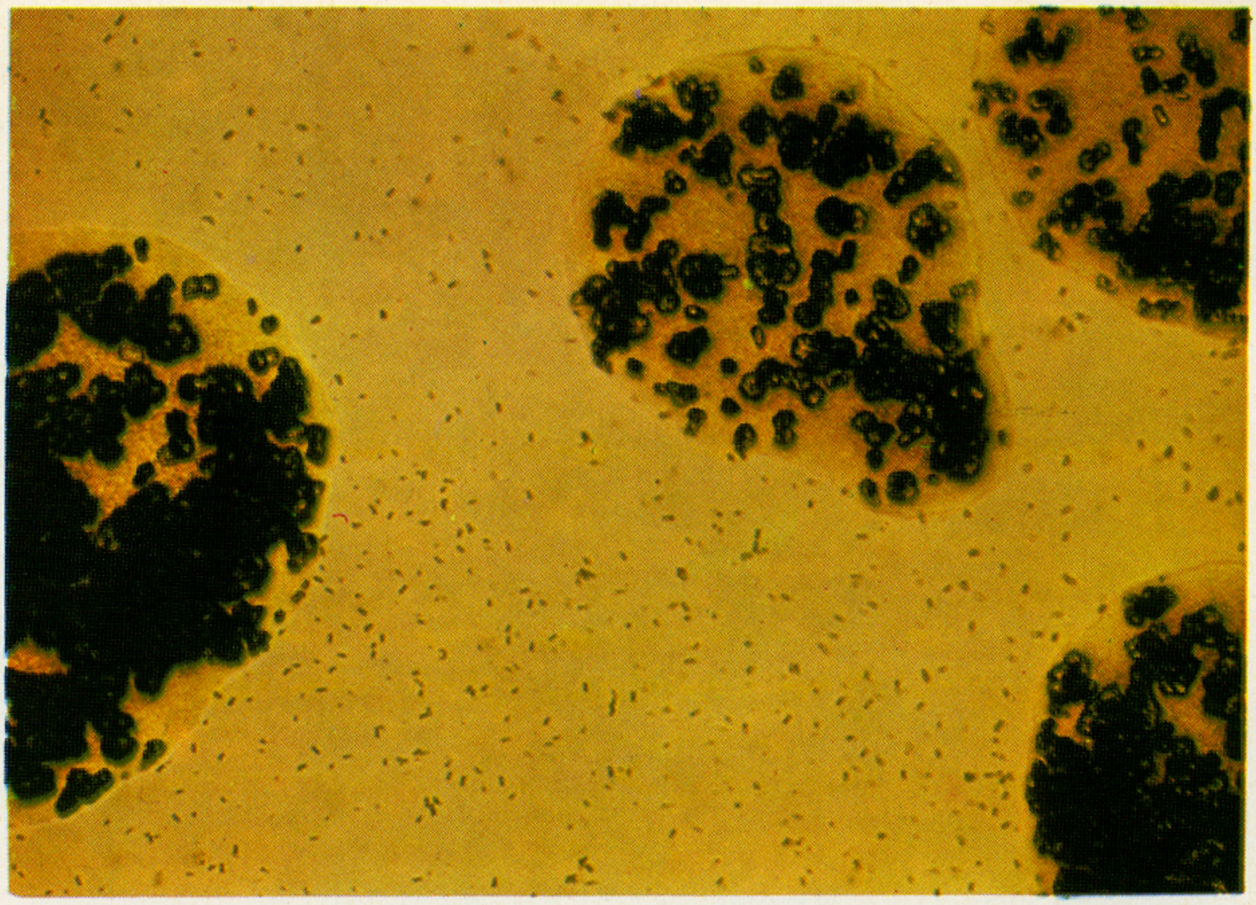


Fot. 1

Nº 2



Fot. 2



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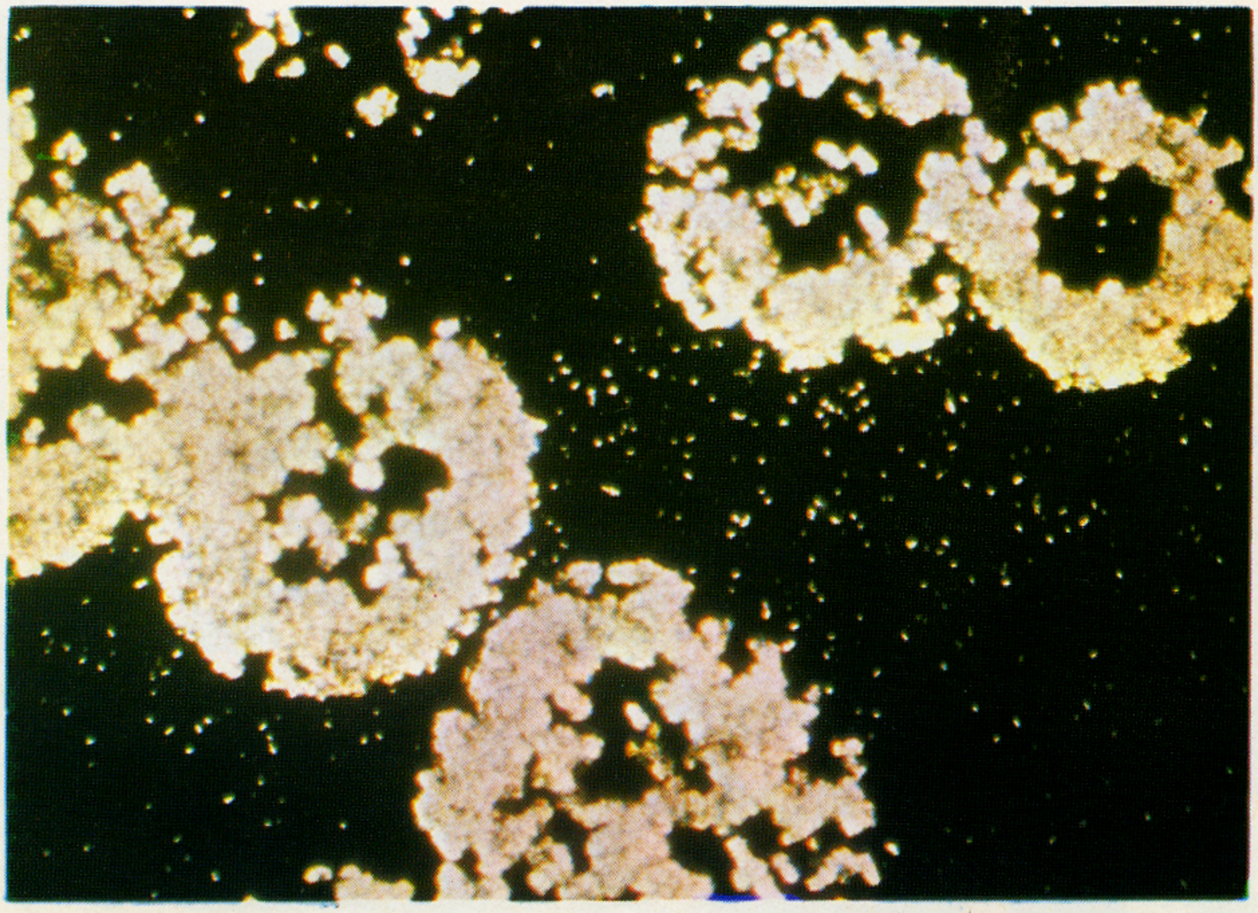

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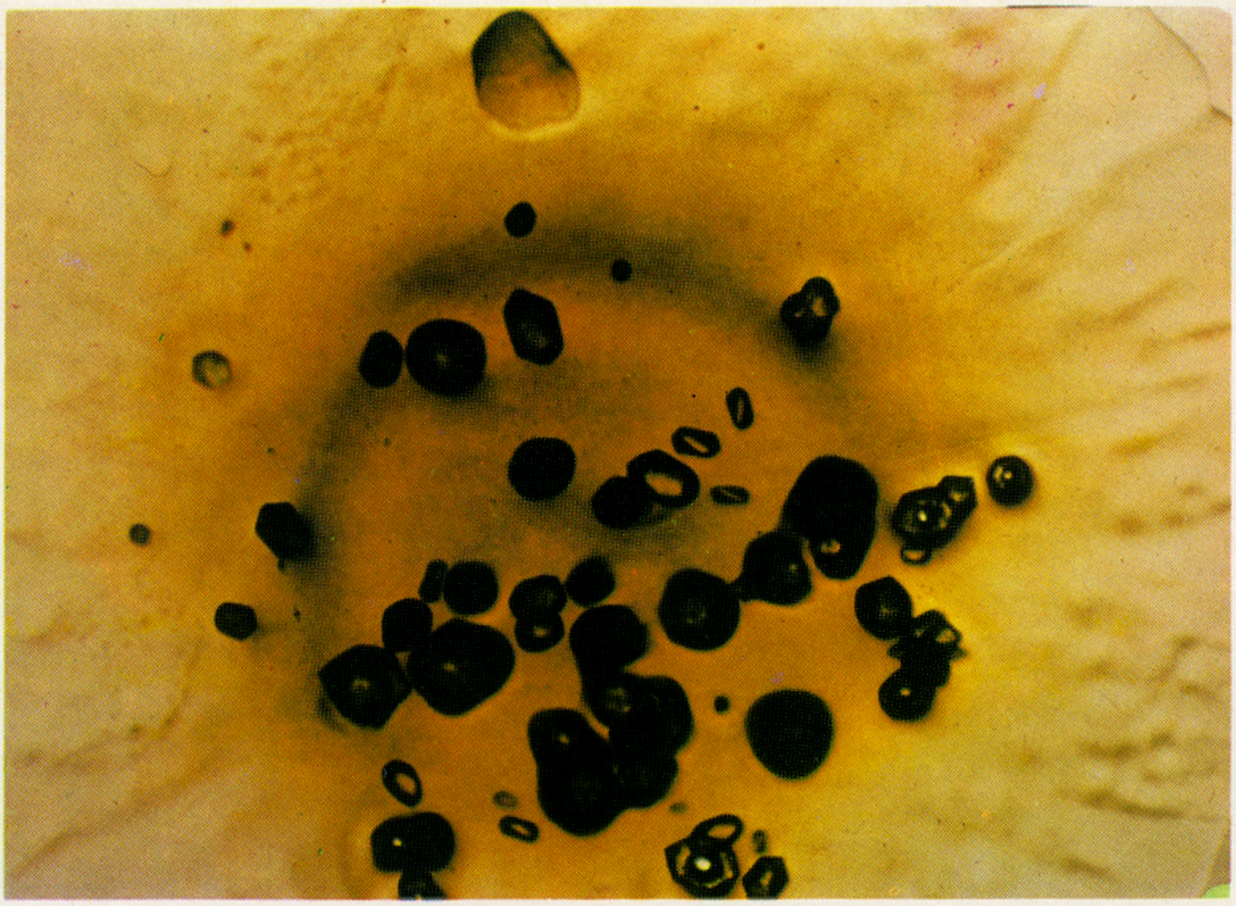
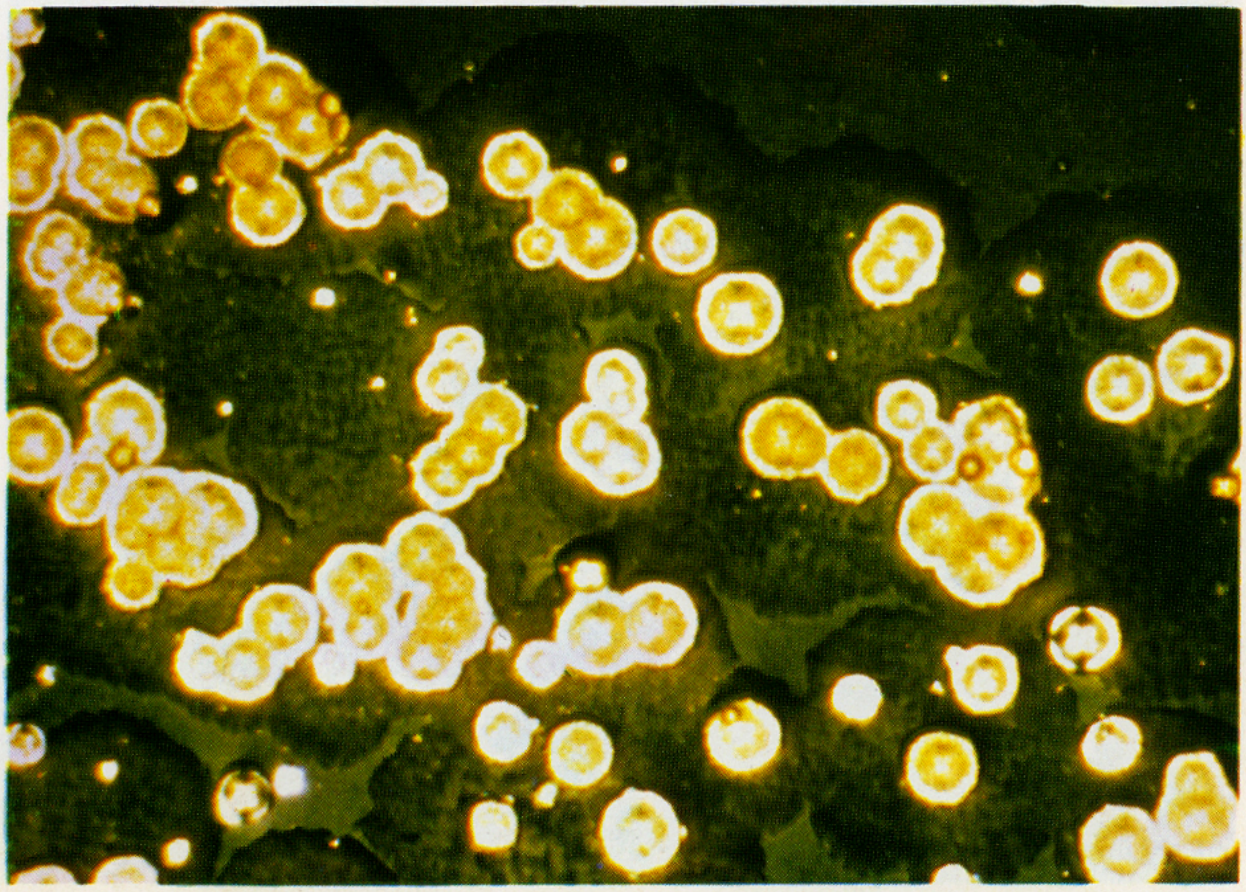
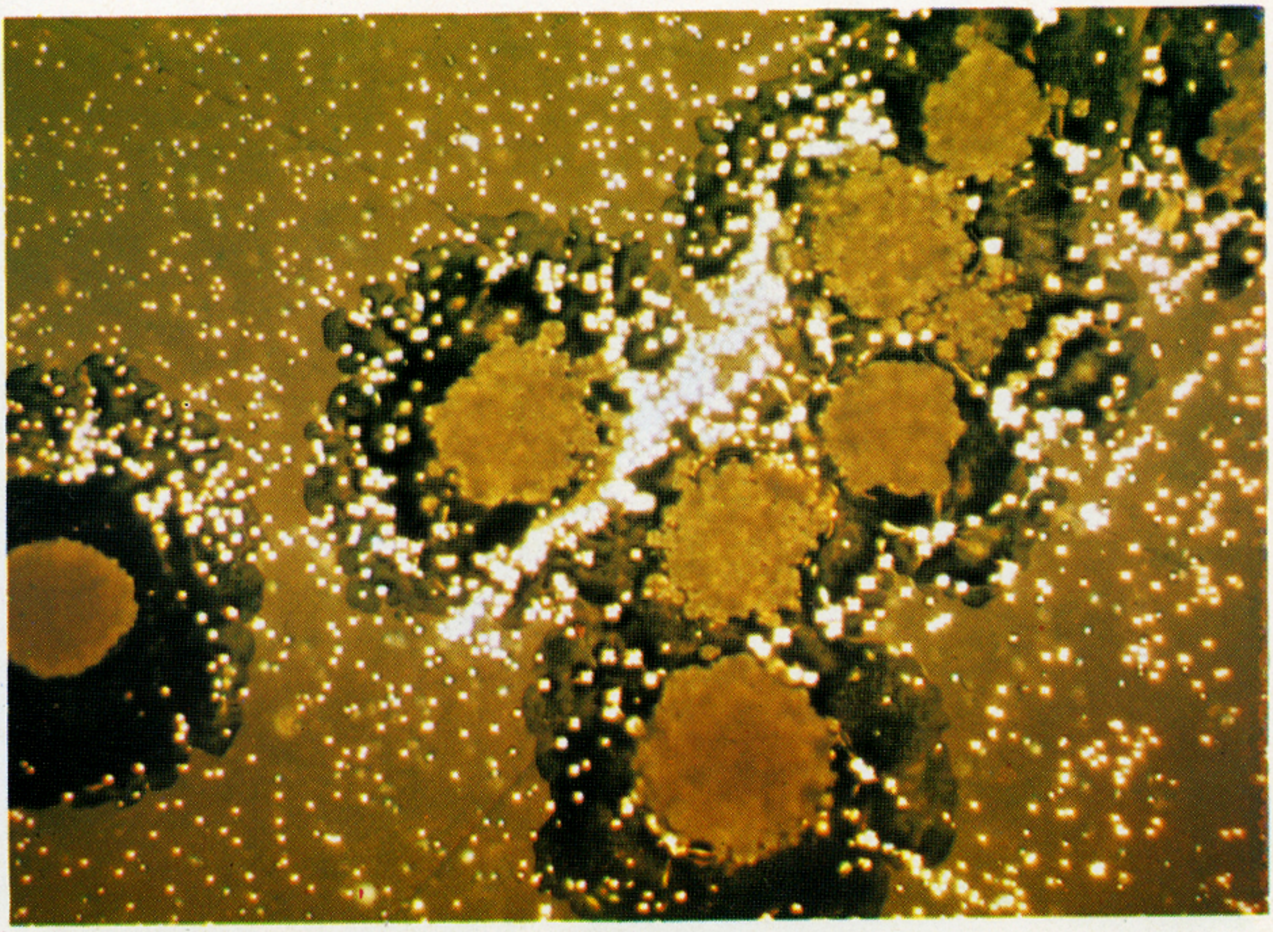


Fot. 5

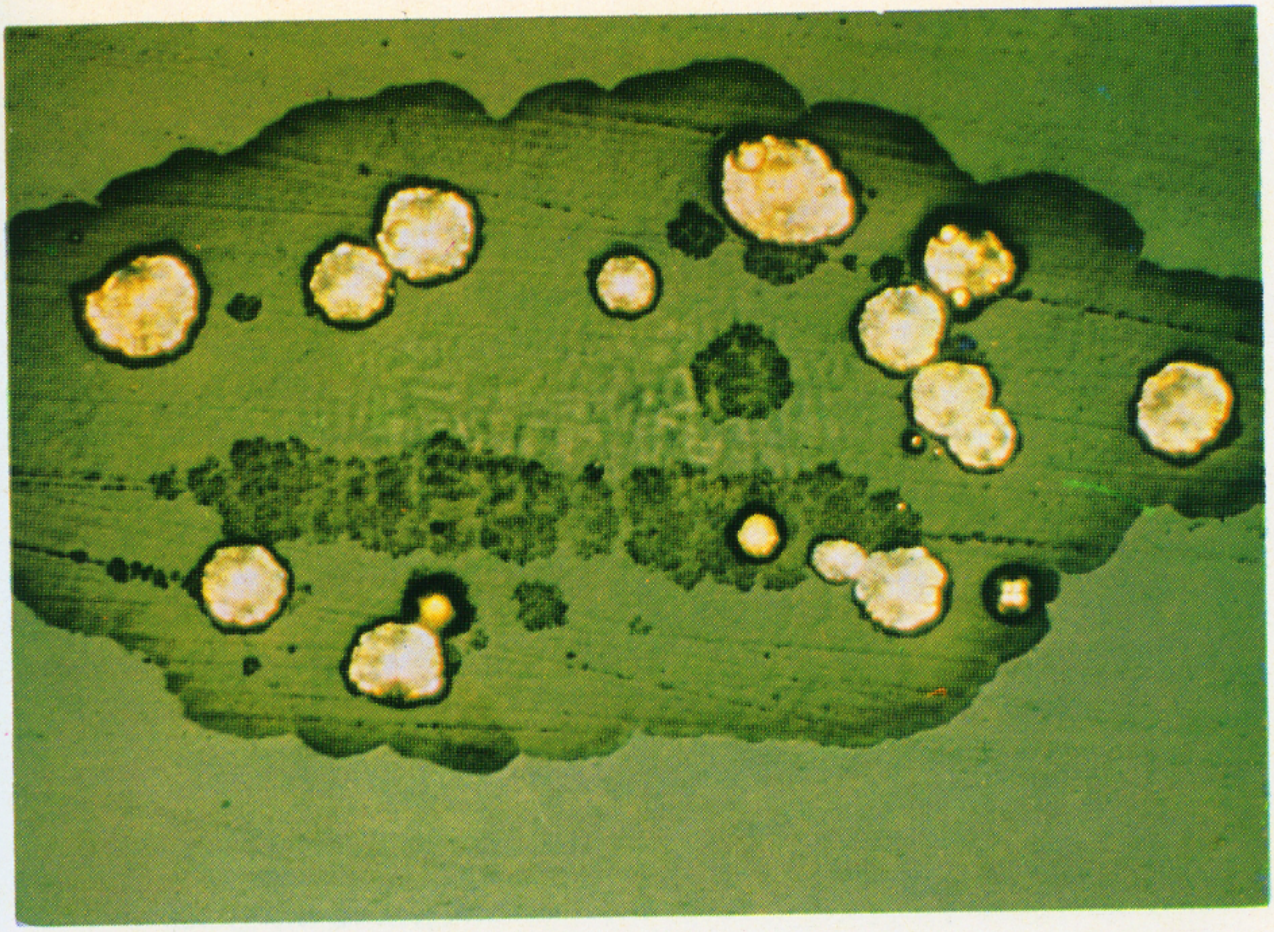


Fot. 6

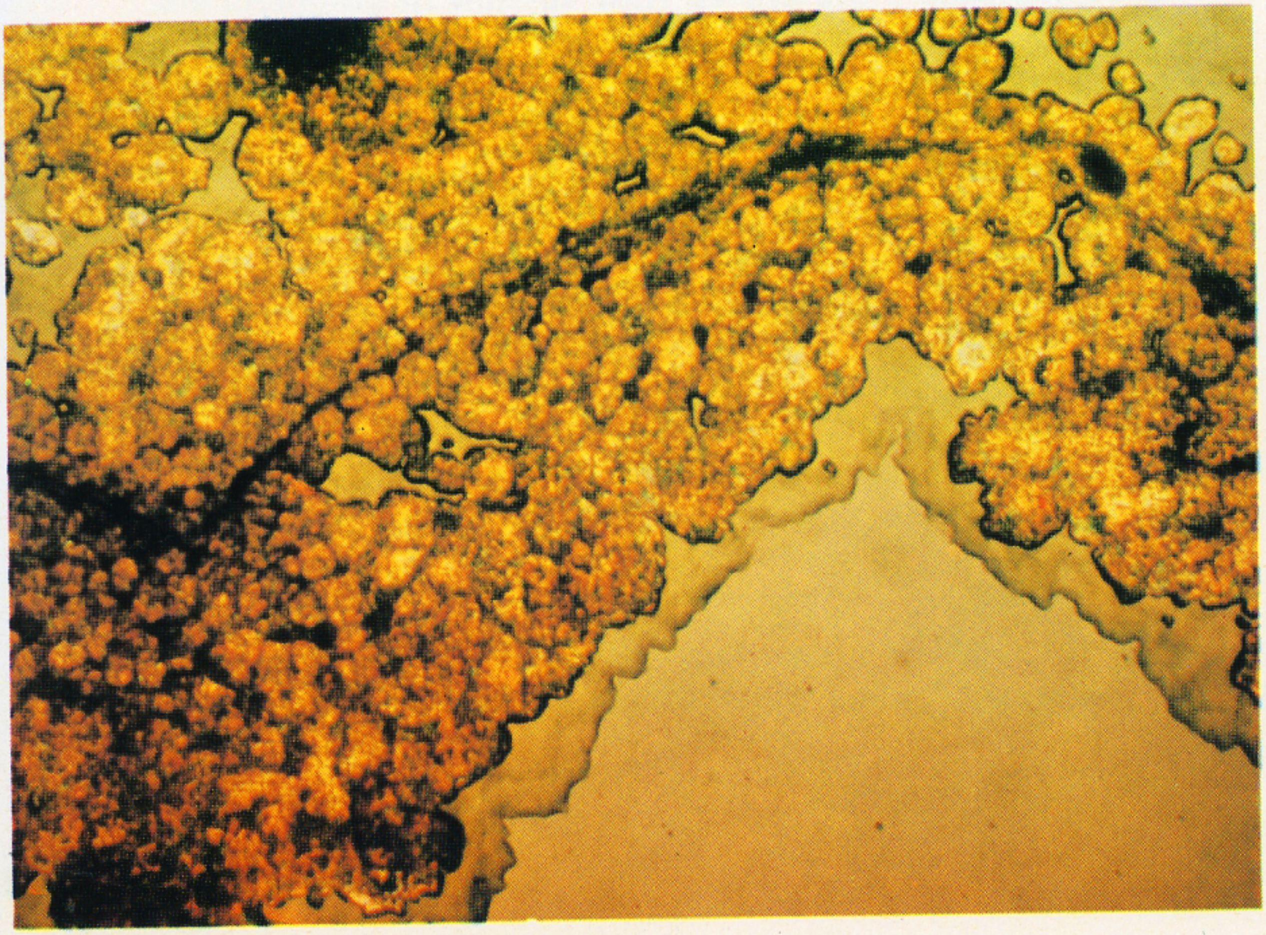
Fot. 7



Fot. 8



Fot. 9



Fot. 10