

A CULTURE MEDIUM OF INORGANIC CHEMICAL SALTS,
COMPARED WITH BOUILLON MEDIA.

Introductory.

The Medium.

Chemical Salts used.

How prepared.

Appearance.

Kind of culture used.

Results noted.

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Synopsis.

Full contents.

Preparation.

Appearance.

Kind of culture used.

Results noted.

Notes.

Used, for, and, results.

Notes.

Preparation of salts.

Kind of culture.

Appearance.

General remarks.

As to results.

As to temperature of culture.

Further investigation is necessary.

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Kind of Culture made
and
Features Noted.

Stab Cultures of Bacteria.

Cereus Flavus.

Ruber Miquel.

Cyanogenes.

Typhosis.

Coli Commune.

Prodigiosis.

Anthraxis.

Fluorescens Non-Liquefaciens.

Sarcina Lutea.

Molds.

Black, Dun, Lemon, Penicillium Glaucum.

Yeast.

Saccharomycetes Roseus.

Plate Cultures.

Conclusion.

General Conclusion.

As to Pigments.

As to Transparency of Medium.

Further Investigation is Necessary.

E. A. DeSchweinitz, Ph.D., M. D., Chief of Biochemic Division, Bureau of Animal Industry, in the sixteenth Annual report of that department, states that in endeavoring to isolate soluble ferments of the hog cholera germ, he had occasion to make use of the following artificial culture medium in which the base is produced from Inorganic Chemical Salts instead of the beef Bouillon generally used.

- Distilled Water - - - - - - - - - -1000 c.c.
- Magnesium Sulphate - - - - - - - - - 0.2 grams.
- Ammonium Phosphate - - - - - - - - - 10. grams.
- Acid Potassium Phosphate - - - - - 1. gram.
- Glycerine - - - - - - - - - -45. grams.
- Agar-Agar - - - - - - - - - -1. %

and Peptonum Siccum - - - - - - - - - -1. % this latter being added upon my own authority.

The report states that the germ of hog-cholera flourishes in this medium and, "It would probably be equally well adapted to many other germs," says Dr. DeSchweinitz. As an incomplete and slight test of the medium above described, several species of bacteria were experimented with. But first we will consider the preparation of the medium.

The Chemical salts were added to the water in the proportions indicated above; the Agar, and the Peptonum Siccum were then added; this compound was boiled for three hours in the hot water bath after which it was neutralized with Sodium Carbonate and then drawn off into sterile test tubes through the hot water funnel, at the same time being filtered through absorbent cotton. The tubes containing the medium were sterilized for three successive days, one half hour per day, in the steam sterilizer.

The appearance of this medium is most beautiful, to the bacteriologist, being nearly colorless and transparent, thus furnishing an excellent opportunity for noting cultural features, especially the growth beneath the surface.

KIND OF CULTURES MADE.

For lack of time and space stab cultures and only a few others will be considered in this thesis and the conclusion will deal with cultural features although some morphologic characteristics were noted.

MICROCOCCUS CEREUS FLAVUS.

Cereus Flavus made a typical growth, the yellow colony spreading out over the surface of the medium and some growth took place all along the needle track. Later (five or six weeks) the entire medium took on a yellowish tint showing that the pigment is soluble in the presence of some of the salts used in preparing the medium. In comparison with Bouillon cultures grown under similar conditions I find that the Bouillon cultures make a much more rapid growth and the pigment does not dissolve in them.

RUBER MIQUEL.

Ruber Miquel made a good growth on the surface of the medium but was not inclined to turn red as in cultures grown on Bouillon compounds although a slight tinge of coloring was to be seen; neither does this bacterium grow as fast on this Chemical Salt medium as it does on Bouillon Media. Below the surface of the medium there was some growth along the needle track, but not extending out any distance.

BACILLUS CYANOGENES. (Blue Milk).

Our cultures already on hand in the Laboratory seemed to have lost their vitality and did not grow, readily, upon any of our various media. However, in the medium under discussion it finally made a reasonable growth and spread itself out over the entire surface of the tube,

taking about three weeks time. There was little or no growth along the needle track; the pigment formed by the bacilli is soluble changing the medium to a bluish-purple hue; this does not occur in Bouillon media and in Bouillon media the growth is more rapid.

BACILLUS TYPHOSIS.

Typhosis also, seemed to have its vitality nearly destroyed in our old cultures and new growths were very slow or impossible to obtain. However, a growth was finally secured; this did not confine itself to any particular portion of the tube but extended all through it. In comparison with growth upon Bouillon preparation, inoculated from the same sources, the results are satisfactory both as to the amount and rapidity of growth.

BACILLUS COLI COMMUNE.

Coli Commune did not develop upon this medium, but this may have been the result of not being well inoculated and will be investigated further.

BACILLUS PRODIGIOSIS (Red Milk).

The bacilli of red milk made a good characteristic growth, covering the surface of the medium in a few days, and following the needle track its entire length. The growth was as rapid as upon media prepared from Bouillon though it may not have been quite as luxuriant. The pigment produced by the bacteria was readily soluble giving a distinct pinkish purple color to the media that was near the bacterial growth. Under the microscope no involution forms were noticed, the bacteria being in every instance similar to those grown on Bouillon compounds.

BACILLUS ANTHRACIS.

The bacilli of anthrax made but little and slow development; the colony did not spread out upon the surface but was confined entirely

to the point of inoculation and seeming to make better growth beneath the surface than elsewhere . But as our anthrax germs did not grow well upon any of our standard media the cause of the slow growth noted above cannot definitely be ascribed to the medium upon which it was grown, but was more probably due to lack of vitality.

STAPHYLOCOCCUS PYOGENES AUREUS.

Pyogenes Aureus grew very slowly; on the surface of the medium the culture spread out only a short distance; along the needle track the growth was apparent, though scanty. The colony didnot acquire the typical color usually found in Bouillon cultures. The latter cultures also grow more rapidly. Microscopically the cocci are identical with those grown on Bouillon media.

BACILLUS FLUORESCENS NON-LIQUEFACIENS.

This bacillus made a typical and rapid growth, covering the surface of the medium with a gray colored colony in four or five days. Immediately after the growth began the medium began to assume a greenish fluorescent appearance near the surface and then extending downward gradually and permeating the entire tube in three or four weeks. The fluorescent tube looks very pretty. The only noticeable difference between the growth on this medium and the growth upon Bouillon media is the scarcity of fluorescence in the Bouillon cultures.

SARCINA LUTEA.

Lutea made a good typical and rapid growth the colony spreading nearly as fast as if grown upon Bouillon media. The pigment formed is soluble in the presence of these salts giving a yellow tinge to the entire media. Under the microscope the Sarcina formation of the cocci is very plain, being as perfect as if grown on a standard Bouillon preparation.

YEASTS.

Saccharomycetes Roseus (Pink Yeast).-- Pink yeast developed excellently upon our artificial medium though not growing rapidly as it does on Bouillon compounds under similar conditions. Its coloring matter is soluble in the presence of these Inorganic Chemicals, giving a pinkish cast to the media immediately in contact with it, a phenomenon which is not so marked in Bouillon cultures.

MOLDS.

Several varieties of molds were grown upon the new culture medium.

BLACK MOLD.

This mold grew more slowly on this medium than is its custom on Bouillon media, yet the growth was typical and to the eye had its usual appearance.

LEMON COLORED MOLD.

This mold made a very slow growth. The organism seemed to want for nutrition. The culture was not typical and would probably not exist for any length of time on such a medium.

DUN COLORED MOLD.

This mold also made a slow growth upon the new medium though it was natural in appearance.

PENICILLIUM GLAUCUM.

Penicillium Glaucum also had difficulty in obtaining sufficient nutrient to cause it to flourish readily; however, its appearance was typical, though the growth was slow and scant.

PLATE CULTURES.

Some plate cultures of various unknown organisms were made with the result of finding the growth upon this medium was generally slower than upon either Bouillon Gelatin, or Bouillon Agar. Two or three of these unknown organisms made growths upon the new artificial medium and

failed to even start upon any other media, but as this was not investigated to any considerable extent the causes for doing so are not known and so no conclusive statement in regard to them can be made.

All of the above cultures were grown at room temperature and without any particular regard to the disposal of the light in the room. In all cases the intention was to have them under the same conditions in which our Bouillon cultures were grown so as to give an equitable basis for comparison.

CONCLUSION.

The investigations carried on seem to point to the following conclusions. Some bacteria, *Sarcina Lutea*, *Micrococcus Cereus Flavus*, and *Bacillus Prodigiosus*, grow almost as well on this medium as on Bouillon preparations, while the various molds experimented with give indications of a lack of the necessary matter for a flourishing subsistence. Pink yeast also grows much slower than on Bouillon compounds.

Generally speaking the medium is not satisfactory for growing bacteria. All pigments tested are soluble in this medium. Such microscopic work as was done showed no involution forms (inconclusive).

The transparency of the medium is a redeeming feature, giving excellent opportunity for noting cultural features.

The investigation was carried forward upon too limited a scale; several times as much work could well be expended upon it.