quinone were observed. However, the amount was too small to make any tests of identity.

The aqueous distillate resulting from the steam distillation of the crude phenols was boiled with an excess of barium carbonate. The only observation worth recording was that the solution became wine colored, a color reaction produced by monohydroxythymoquinone under like circumstances.

It may be worth while to point out that of the 45 Gm. of crude thymol separated in crystalline state from the oil, 40 Gm. were obtained from the ether[4] with which the alkaline phenylate solution had been shaken, and only 5 Gm. from the oily phenols separated from the aqueous phenylate solution by acid. Hence, of the 134 Gm. of phenol indicated by assay, only 45 Gm. or 33.5 percent crystallized out and were characterized as thymol. No small amount of phenol separated as "tar."

Non-Phenols.—The small amount of non-phenol portion of the oil was likewise fractionated. Below 177° only 10 Cc. came over, and between 177° and 235° less than 20 Cc. From the residue in the flask some hydrothymoquinone was separated as described under "phenols" and identified as such.

Conclusions.—Although the amount of oil was too small to accomplish much, nevertheless this preliminary investigation has added at least one substance to the list of known constituents of *Monarda punctata, viz.*, hydrothymoquinone.[5] Reasoning by analogy with *Monarda fistulosa*[6] seemed to show conclusively that it must be present in the plant, but its isolation had heretofore escaped all efforts. Moreover, the presence of monohydroxythymoquinone is indicated by a color reaction and that of dihydroxythymoquinone but wants definite chemical identification. Hence, this minor diversion from a major problem seems fully justified, and if only for the reason that it will stimulate the efforts to secure a much larger amount of material next year and thus take another step in further establishing the interesting parallel in the biochemistry of *Monarda punctata* and *M. fistulosa*.

COMMERCIAL CULTURES OF BULGARIAN BACILLUS.*

BY EDGAR B. CARTER.

A short historical sketch opens the paper to show that Metchnikoff did not originate the theory of the harmfulness of the absorption of bacterial toxins from the intestines nor was he the first to suggest the use of the Bulgarian Bacillus in the treatment of these conditions. The bacteriological characteristics and the chemical

[4] The fact that thymol, dissolved in aqueous sodium hydroxide, can subsequently be shaken out with ether does not seem to have been reported in the literature before, although this phenomenon was observed by Jahns in connection with carvacrol. *Ber.*, 15, 817.

[5] It may be worth mentioning that hydrothymoquinone has been precipitated from ordinary, colorless, *Monarda punctata* oil by Dr. Edward Kremers by merely diluting the oil with the requisite amount of heptane; also that a number of previously unknown constituents have been isolated and identified by Dr. N. Wakeman. (See *Circulars of Wis. Pharm. Expt. Station.*) In both cases, however, the results had not been published at the time of this writing.

[6] I. W. Brandel, *Pharm. Rev.*, 19, 244.

* Read before Scientific Section, A. Ph. A., Chicago meeting, 1918.

changes produced in milk by the organism are taken up briefly, after which the three forms of commercial cultures are discussed. The advantages and limitations of each are shown. The paper closes with a description of the tests used to establish the values of the cultures with a simple test by which the pharmacist may satisfy himself as to their viability. A bibliography follows.

Philosophy is a very potent force in scientific work; it reasons far ahead of the known into the realm of the unknown, thereby furnishing not only an incentive for original work but assisting in the formulation of systematic schemes by which this work may be directed. In 1901, when Eli Metchnikoff, in a public lecture at Manchester, England, announced that his investigations had led him to the conclusion that the lifetime of mankind is limited largely by the absorption of toxins and the products of putrefaction from the bowels, he laid the foundation for all his subsequent work in which he attempted to discover the means of eliminating or at least decreasing this intestinal putrefaction, with a view to prolonging life for a number of years.

While we cannot feel that the work of Metchnikoff has to any extent solved the problem of the prolongation of life, he himself dying at the age of seventy-one, there can be no doubt but that his philosophy is sound and that possibly a younger scientist may take up the same work and ultimately achieve this great man's object.

There is probably no doubt but that a great many of the conditions appearing in man in which symptoms profoundly affect the nervous system, the circulation, and in a variety of ways the entire body, can be clinically traced to the intestines. As Metchnikoff pointed out and demonstrated, the absorption of these putrefaction products of proteins—the phenols, skatol and indols—produce degenerative changes in the arteries, kidneys, liver and digestive organs.

It is interesting to note that a few years later, the writer of the famous Craig Kennedy stories in the *Cosmopolitan* took for the plot of one of the crimes, in which the famous Craig brought the criminal to justice, the gradual but progressive poisoning of a rich husband by his young and beautiful wife, in which the dastardly deed was accomplished by secretly administering indol in the man's food and drink. It made an excellent and exciting story, although a very improbable one when we consider the strong and obnoxious fecal odor of indol, but no doubt it would have done the work if the man could have been forced to eat it.

Metchnikoff was not the first investigator to consider the harmfulness of the absorption of putrefaction products from the bowels nor was he the first to suggest a remedy. Since the trouble resulted from the bacterial decomposition of protein, it was quite natural that the early workers should attempt to combat the condition by adding acid-producing bacteria and carbohydrates to the diet on the basis of antibiosis or mutual antagonism which they had learned existed—cultures between many acid-producing bacteria and the saprophytic group. So in 1886 we find Escherich[1] and Brudzinske[2] proposing the use of *B. lactis aerogenes* and in 1887, Quincke[3] suggesting the use of the yeast *Oidium lactis*. It was not, however, until 1906 that Metchnikoff[4] suggested the use of the Bulgarian Bacillus which had previously been isolated and studied by Massol,[5] Cohendy[6] and Bertrand and Weisweiller.[7]

BACTERIOLOGY.

The *Bacillus Bulgaricus* (*B. Massol*) is a non-pathogenic, non-motile, non-sporulating, non-liquefying, Gram positive bacillus (involution forms are Gram negative) showing wide variations in lengths, from 2 to 50 microns and occurring singly or in short and long chains. It is both an aerobe and anaerobe, growing with difficulty on ordinary media, but easily cultivated in milk or in medias containing whey or malt. It grows best at 44° centigrade, fairly well at 30°, slightly at 25°, but not at all at 20°.

CHEMISTRY.

The *Bacillus Bulgaricus* belongs to the lactic acid-forming class of bacteria and in common with the *B. lactis aerogenes*, *streptococcus lacticus* and others, splits the hexoses of the monosaccharides into two molecules of lactic acid. In milk cultures the lactose is hydrolyzed and converted into galactose and glucose. The *Bacillus Bulgaricus* probably has nothing to do with the primary hydrolysis, for it does not form acid from lactose in the culture tube nor does it grow very well in media made with lactose. Not only is the bacillus resistant to high acid concentration but it is a vigorous acid producer, forming from 2 to 3 percent of acid in suitable media. While it is customary to speak of the acid resulting from the splitting of the sugars by the *Bacillus Bulgaricus* as lactic acid, Bertrand and Weisweiller[7] have shown that acetic and succinic acids are also produced in appreciable quantities. Recent work of Van Slyke and Baker[8] indicates that only a small part of lactic acid remains free in milk cultures. Immediately upon its formation, the lactic acid begins to combine with the basic phosphates and citrates of calcium, magnesium, sodium and potassium, forming monobasic or acid phosphate and citrate salts. When these reactions are complete, the acid combines with the calcium caseinate[9] forming calcium lactate and free casein. Hence the formation of the curd.

COMMERCIAL PREPARATIONS.

It is not within the scope of this paper to discuss the indications for the therapeutic use of Bulgarian Bacillus. We shall leave that to the clinicians. The preparation has been used for the treatment of practically every disease ranging from diabetes to fetid perspiration, but since all good things are carried to extremes by the enthusiast, we should not be surprised that some absurd claims should be made for Bulgarian Bacillus. It is sufficient to say that there is and will doubtless continue to be a very steady and increasing demand for preparations of Bulgarian Bacillus from the medical profession, who are surely separating the wheat from the chaff. It is for the pharmacist and pharmaceutical chemist to furnish them with dependable and potent products.

So far, the general use of Bulgarian Bacillus cultures has been limited to three forms—first, the liquid cultures; second, the tablets; and third, the suspension in a medium such as petrolatum—the problem being that of making accessible to the doctor massive doses of viable bacilli.

It is evident that the maximum doses of the bacilli can be obtained by the administration of the straight liquid culture in which the media is practically filled with bacterial bodies. Naturally such a culture, after incubation, must

be kept at a cool temperature—below 20° centigrade (68° F)—or the bacilli will continue to grow and soon exhaust themselves, so while liquid cultures require greater care, there can be no doubt but that they possess greater counts and if properly cared for should have a very rapid and very potent effect. The liquid cultures are prepared by growing the bacilli in sterile liquid medias, preferences being given to some variation of the Cohendy media. This consists of slightly alkaline whey to which has been added gelatine and cane-sugar. The growth is controlled by titrating the acidity and by actual bacteria count, and is stopped just short of an acidity which experience has taught us is detrimental to the life of the bacilli.

Before marketing it is tested bacteriologically to establish the identity of the culture and to assure the absence of contaminating microörganisms. The better laboratories also test each lot to determine its actual power to inhibit the growth of *bacillus*

The tablets are prepared from milk or other liquid cultures, mixed to a mass with milk sugar, starch, or both, dried and formed into tablets. Although the bacilli are materially diluted by the powder necessary to form a tablet, their vitality is preserved over a much longer period of time and under more adverse temperatures. They are usually tested by dropping one tablet in a pint of sterile milk and incubating to obtain the souring of the milk with the characteristic curd in twenty-four hours.

Another method of preserving viable Bulgarian Bacilli is that of drying the culture at a low temperature and mixing them with an inert substance such as petrolatum. The petrolatum is generally colored red to minimize the effect of the light on the bacilli. This method, although in use for a number of years, was patented last March.[10] There can probably be no doubt but that this method is the most effective one known to-day for preserving the bacilli, but one cannot help but wonder if the protection afforded by coating the bacilli with mineral wax is not sufficient to prevent their later growth and multiplication in the intestines.

TESTS OF BULGARIAN PREPARATIONS.

Because of the fact that Bulgarian Bacillus can only be grown in a proper incubator and because of the fact that media used, which if contaminated will grow pathogenic bacteria abundantly, thereby making a dangerous instead of a beneficial culture, it would seem that the preparation of Bulgarian Bacillus cultures had best be left to the properly equipped biological laboratory. Bulgarian Cultured Milk, however, can be prepared very nicely in a drug store equipped with a fireless cooker, as the soft, smooth curd which results from the growth of Bulgarian Bacillus is very distinctive, as are the flavor and acidity. Bulgarian Cultured Milk can only be made of milk which has first been rendered sterile by boiling for a few minutes.

Even though he makes no attempt to grow the cultures, it is possible, however, for the pharmacist to make simple tests which will aid him in determining the value of the various cultures in which he is interested. The only absolute scientific tests of the cultures are the plating tests in which definite quantities are inoculated into suitable media, poured into petri dishes, and the number of typical colonies that develop, counted. Such a procedure can only be carried on by a trained bacteriologist.

Some men have attempted to judge the viability of these cultures by examinations for motility under the microscope, forgetful of the fact that the Bulgarian Bacillus is non-motile. Others have attempted to stain a smear of the culture and have also met with failure because there is not sufficient albuminous material in the media to properly fix the bacteria to the slide. If a few drops of the culture be mixed with a large drop of egg white or Mayer's Albumin Fixative and the two well mixed and smeared, if this smear be dried, fixed by heat, stained by Gram's method and examined under the high power of the microscope, a fairly good estimate may be made of the number of viable bacilli. All dead bacilli stain a Gram negative while those which are still viable are Gram positive. This interpretation will have to be modified in case the involution forms appear, as they generally stain as negative, even when living and capable of growth.

It is needless to say that the majority of the druggists to-day are making strong efforts to furnish their trade with potent biological products and are providing facilities for the storage of perishable articles at a reasonably low temperature; and it is the loyal coöperation of this class of pharmacists that makes possible the marketing of dependable cultures of Bulgarian Bacillus.

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ANALYSES OF MILK OF MAGNESIA.*

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Due to the wide variation of the declared content of magnesium hydroxide in the various standard brands of Milk of Magnesia, and the silence of other manufacturers as to the content, the writer became interested to know how many of these standard brands conformed to the U. S. P. IX or to the former standard, the N. F. III. It was with this object in view that these analyses were made.

Table A shows the percentage of Magnesium Hydroxide in the leading seven brands and five control samples—one prepared according to the U. S. P. IX, one according to the N. F. III, one according to Beringer's process[1] modified by

* Read before Section on Practical Pharmacy and Dispensing, A. Ph. A., Chicago meeting, 1918.

[1] Geo. M. Beringer, Proc. N. J. Ph. A., 1913, pp. 46–48.