



FIG. 1. An improved sodium burner, showing: I—Side view of the complete burner with one side wall cut away to expose the vibrating mechanism (D) and funnel (C) containing salt; II—Front view of chimney (A), of Meker type, and attached mixing chamber (B), into which gas, air and powdered sodium chloride flow before combustion. (Drawn to scale and reduced to about one fourth of actual dimensions.)

nickel grid inside a cap, 30 x 55 mm at the rectangular top. Fixed under the salt funnel is the vibrating unit

(D), connected by five feet of insulated wire to the press button (F), and to a battery (G) of one or two dry cells. The entire burner is clamped on a short iron stand which permits adjustment for height.

In order to operate the burner the funnel is first filled with salt and capped. Air is then blown through the mixing chamber to displace any salt which may have fallen in during the filling of the funnel. The air is turned off and the gas is ignited to give a final flame of the desired size. Air is then introduced to give the most efficient combustion of the gas. The burner is placed with the front toward the polariscope. A short period of action of the vibrating unit causes the salt to flow into the mixing chamber from whence it is carried up by the forced draft of air and gas and burned in the flame.

As a sodium salt we have found the chloride, finely powdered for 24 hours in a ball mill and dried at 110 degrees C., most satisfactory, although the anhydrous powdered sulphate has also given an intense light. The burner may be used to produce monochromatic lights from non-hygroscopic salts of other metals. The size of the flame may be reduced for spectroscopic studies. If operated for long periods of time, particularly without critical adjustments of gas and air, the burner should be placed in a well-ventilated hood for removal of fumes.

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SPECIAL ARTICLES

THE PRESENCE OF BACTERIA WITHIN THE EGGS OF MOSQUITOES

IN connection with certain experiments on the food of mosquito larvae (to be described elsewhere) the investigator was astonished to find evidence of viable organisms within the ova of *Aedes aegypti*. Six eggs, after a thorough disinfection in hexyl resorcinol,¹ were introduced into sterile 0.5 per cent. dextrose bouillon and the cultures incubated at 80° F. In one instance a larva hatched after 48 hours and the media remained sterile for 9 days, at the end of which time a second larva hatched and within 24 hours a spontaneous contamination appeared in the culture. Throughout the course of the experiment the plug was not removed from the flask. This sudden appearance of bacterial growth could not be explained on the basis of a slow growing organism, otherwise a heavy growth would not have suddenly arisen in a flask which had previously remained perfectly clear for a period of nine days. This first observation was made in January, 1931, and since then in several hundred experiments, with different types of media, only seven

additional clear-cut instances have been found. The length of time these eight cultures remained sterile, previous to the appearance of contamination, varied from 5 to 11 days, the average being 9 days. The organisms isolated include the following—gram negative and gram positive bacilli, staphylococci and yeast.

It is only in cases where the hatching of certain of the eggs is delayed for several days that this phenomenon could be demonstrated. If the contamination had arisen within the first 72 hours or had appeared slowly it might be assumed that the exterior of the eggs had not been sufficiently sterilized or their introduction into the media not carefully executed. It is felt that in many such cases where contamination did appear promptly this may be accounted for by the presence of bacteria in the eggs. To the writer only one explanation of the above quoted experiments seems tenable—namely, that bacteria were present in the eggs which were delayed in hatching and upon emergence of the larva the bacteria multiplied rapidly in the bouillon. In media other than that suitable for rapid growth of bacteria there could be no indica-

¹ E. H. Hinman, *Amer. J. Trop. Med.*, 12: 263, 1932.

tion as to when the contamination arose, but in bouillon it becomes apparent immediately.

HISTOLOGICAL STUDY OF CULICID EGGS

In order to test the above-mentioned hypothesis a considerable number of eggs of the following species of mosquitoes have been sectioned—*Aedes aegypti*, *Anopheles quadrimaculatus*, *A. crucians* and *Culex quinquefasciatus*. The eggs were fixed in Bouin's fluid, very gradually passed up through the alcohols during the course of a week, cleared in cedarwood oil, passed through xylol and infiltrated and imbedded in paraffin. The eggs were allowed to orientate themselves at random in the paraffin, but of course were all in the same horizontal plane so that longitudinal sections could readily be obtained. Several hundred eggs were imbedded in a block and in the case of *Culex quinquefasciatus* a number of egg masses (200 to 500 ova per raft) were placed in each block. Altogether several thousand eggs have been sectioned, cut 5 μ or less in thickness. Different stains have been tested, including iron hematoxylin, methylene blue, acid-fast and gram stain. In a few slides a very delicate hematoxylin stain was first applied, followed by a bacterial stain. The gram stain alone has proven most satisfactory.

Microscopical examination of these sections has revealed the presence of bacteria in a number of slides. Probably only a relatively small percentage of eggs actually contain organisms, as would be indicated by both cultural experiments and histological study. The most common type of bacteria encountered in the sections was the coccus, but on occasion streptococci have been found and also other cocci showing evidence of division. Bacilli were very rare and in no case were large numbers found in any egg.² The difficulties involved in the demonstration of bacteria in tissues are well known and in a number of instances organism-like structures were observed, but because of indefiniteness of staining, irregularity of outline or size there was considerable hesitation in regarding these as true bacteria.

From a careful examination of over 250 slides the writer feels assured that bacteria occasionally occur in mosquito ova. Unless large numbers of eggs had been used, the results might well have been negative.

The possibility of hereditary transmission of the etiological agent of either yellow fever or dengue through the mosquito host is of great epidemiological importance. To date experimental work along these lines has been negative, with a single doubtful exception. Yet if viable bacteria may occasionally be recovered from the ova of *Aedes aegypti* one might expect that the virus of either yellow fever or dengue

² The writer is greatly indebted to Colonel C. F. Craig for his examination of certain of these sections and appreciates his valuable opinion in this matter.

would, under certain circumstances, appear in the eggs of infected females.

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THE IDENTITY OF STREPTOCOCCI OF ANIMAL ORIGIN WITH CERTAIN STRAINS OF STR. EPIDEMICUS¹

STR. EPIDEMICUS was originally described by Davis² as the etiological factor in milk-borne epidemics of septic sore throat. This organism differed from ordinary hemolytic streptococci in that it produced distinct capsules, produced large, moist colonies on blood agar and was less actively hemolytic than the usual strains of *Str. hemolyticus*. Brown, Frost and Shaw³ in a study of the beta streptococci in milk found certain strains of streptococci which produced a low acidity in glucose broth, failed to hydrolyze sodium hippurate, caused complete hemolysis in a fluid medium, produced capsules, and gave large, moist colonies on blood agar. These strains produced acid from lactose, sucrose and salicin while they did not attack mannite. Such cultures were referred to as *Str. epidemicus* and classified as streptococci of human origin.

In a previous paper⁴ we have shown that there exists among the lower animals a great group of hemolytic streptococci possessing the characters attributed by Brown, Frost and Shaw³ to human streptococci. It is apparent that streptococci possessing the characters of the human type of Brown, Frost and Shaw can no longer be considered to be of strictly human origin. Furthermore, it was demonstrated that 96 per cent. of the streptococci of animal origin could be differentiated from human streptococci by their action on sorbitol and trehalose. Of 40 human cultures tested, all fermented trehalose and failed to attack sorbitol. Of 125 animal strains examined, 120 produced acid from sorbitol but failed to ferment trehalose. The remaining five animal strains have not as yet been successfully differentiated from the human streptococci.

Since the publication of our first paper⁴ it has been found that the group of 120 animal strains referred

¹ The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the director.

² D. J. Davis, "Bacteriologic Study of Streptococci in Milk in Relation to Septic Sore Throat," *Jour. Amer. Med. Assoc.*, 58, 1852. 1912.

³ J. H. Brown, W. D. Frost and M. Shaw, "Hemolytic Streptococci of the Beta Type on Certified Milk," *Jour. Infect. Dis.*, 38, 381, 1926.

⁴ P. R. Edwards, "The Biochemical Characters of Human and Animal Strains of Hemolytic Streptococci," *Jour. Bact.* In press. 1932.