

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. EPIDEMICUS*¹

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.

to above regularly produce capsules under suitable conditions. Furthermore, on the blood-ascitic fluid agar of Pilot, Hallman and Davis⁵ these cultures produce large, moist, surface colonies and spreading colonies between the agar and glass.

The group of 120 cultures has been compared with 16 cultures of *Str. epidemicus*.⁶ The two groups have been found identical in every respect. They produce a low acidity (not exceeding pH 4.8) in glucose broth, fail to hydrolyze sodium hippurate, produced a marked hemolysis in fluid mediums, and under suitable conditions produce large, distinct capsules. On ascitic fluid-blood agar they produce large, moist colonies. They ferment lactose, sucrose, salicin and sorbitol. Mannitol and trehalose are not attacked.

From the results cited above it is evident that, by the methods now in use, it is not possible to differentiate the cultures of *Str. epidemicus* in question from streptococci of animal origin. The group of animal strains which we have studied is composed of cultures isolated from horses, chickens, hogs, cows and foxes. This type is the predominant one in animals and, with the exception of the high-acid-producing strains from bovines and *Str. equi* of strangles, comprises approximately 95 per cent. of the hemolytic streptococci of animal origin which we have studied.

Further comparative studies are being carried out with cultures identified as *Str. epidemicus* in other laboratories. The results of these studies will be reported later.

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THE CAROTENE CONTENT OF TEN VARIETIES OF CARROTS

LITTLE is known of the variation in carotene content of different varieties of carrot, other than the fact that the roots of certain varieties look yellower than others. For this study ten varieties, comprising white, yellow and orange garden and field carrots, were grown under greenhouse conditions. Seeds were planted in November, and the plants were harvested 23 weeks later.

For analysis, the roots were chopped, and a 50 gram sample was ground with sand. Without being dried, the sample was exhaustively extracted with cold acetone, and the extract was shaken with 1 volume of petroleum ether and 2 volumes of 2 per cent. NaCl. The pigment passed completely into the petro-

leum ether layer. The aqueous acetone layer was discarded and the petroleum ether was washed 4 times with the salt solution.

The petroleum ether extract was compared spectrographically with a weighed amount of purified carotene, which had been put through the same "extraction" procedure as the carrot roots themselves. Comparison of the characteristic blue-violet absorption bands photographed in the series of known and unknown dilutions gave the data from which the carotene content was estimated.

TABLE I

Variety of carrot	Carotene content	
	mg per 100 gm	gm per bushel
Early Scarlet Horn	9.6	2.2
Oxheart (Guerande)	8.9	2.0
Long Orange	8.3	1.9
Danvers Half Long	8.2	1.9
Chantenay	8.0	1.8
Earliest Scarlet Forcing	6.4	1.5
Large Yellow Belgian	2.9	0.66
Isbell's Victoria	1.5	0.34
Large White Belgian	0.15	0.034
Isbell's Maude S	0.12	0.027

The findings are summarized in Table I, from which it is evident that the garden varieties contain the largest amount of carotene, although traces are found even in the white field carrots. The extremes in carotene content are shown by Early Scarlet Horn and Isbell's Maude S, the former having 80 times as much as the latter. It is an interesting exception to the common preference for colorless foods that the varieties of carrot most highly esteemed for human consumption are those containing the most carotene.

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⁵ I. Pilot, B. Hallman and D. J. Davis, "A Culture Medium for the Isolation of *Str. epidemicus*," *Jour. Amer. Med. Assoc.*, 95, 264, 1930.

⁶ These cultures were kindly furnished us by Dr. W. D. Frost. The group is composed of cultures isolated from the udders of cows and from epidemics of septic sore throat in humans.