Nile blue is tolerated well in the weaker solutions up to 0.5%. The endoplasm takes a pale blue color; the ectoplasm appears much paler, with a greenish hue. This latter point was not consistent, and when used in combination with neutral red does not have any particular advantage as a contrast stain.

Neutral red and Janus green are the most satisfactory dyes used, so far. The endoplasm and ectoplasm are more clearly differentiated and the fact that the nucleus with its chromatin arrangement is more visible may have some clinical value.

5260

Bacteriology of the Liver in Normal Dogs.*

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The presence of organisms in the tissue of normal, healthy animals has been a controversy for some time. However, investigators have definitely proved that the tissues of normal, healthy animals harbor organisms. That the occurrence of organisms in normal tissue in the experimental animal has been a source of incorrect observation and deduction in certain experimental problems has recently been shown by Ellis and Dragstedt.[1]

C. B. Hawn,[2] working with Holmes Jackson, first described a spore-bearing bacillus found in the liver of normal dogs. Walbach and Saiki[3] studied this bacillus, using 23 normal, healthy dogs, and found this gram-negative, spore-bearing bacillus in 21 of the 23 dogs. Berg, Zan, and Jobling[4] cultured the liver from 11 normal dogs and found this gram-negative, spore-bearing organism in 100% of their animals. Ellis and Dragstedt,[5] in their investigations on liver autolysis *in vivo,* encountered the same organism in almost 100% of their animals.

We studied the bacteriology of the contents of the stomach, the duodenum, and the liver in a series of normal, healthy dogs and the

* Aided by a grant from the David Trautman Schwartz Research Fund of Tulane University.

1 Ellis and Dragstedt, *Arch. Surg.*, 1930, **20**, 8.

2 Hawn, C. B., quoted by Wolbach and Saiki.

3 Wolbach and Saiki, *J. Am. Med. Assn.*, 1909, **21**.

4 Berg, Zan and Jobling, PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 433.

5 Ellis and Dragstedt, *Arch. Surg.*, 1930, **20**, 8.

bacteriology of the stomach and the liver in 4 normal, healthy puppies, 8 days old. All specimens for culture were obtained under strict aseptic conditions. The material obtained from the animals was cultured aerobically and anaerobically in digest meat broth at 37.5°C. The positive cultures were studied by smear methods stained with gram stain after 24 and 36 hours' incubation.

The *liver* was cultured aerobically and anaerobically from 40 dogs. There were positive cultures in 37 dogs, negative cultures in 3. Gram-negative, spore-bearing bacillus was found in 34 of the dogs, giving a positive culture in 92.5% and the presence of a gram-negative, spore-bearing bacillus in 85% of the cases. The remaining 6 cases showed the presence of gram-positive, gram-negative bacilli and gram-positive diplococci.

Contents of the *stomach* were cultured 16 times out of a series of 20 dogs. A positive culture was obtained in 15 instances, and a negative culture in 1 case. In 10 instances the presence of a gram-negative, spore-bearing bacillus was demonstrated. This gives a positive culture of the gastric contents in 93.7% of the animals and the presence of a gram-negative spore-bearing bacillus in 62.5%.

Contents of the *duodenum* were cultured in 19 out of the 20 dogs. Duodenal contents gave a positive culture in 17 cases, or 89.3%, negative cultures in 2 of the animals, and the demonstration of a gram-negative, spore-bearing organism in 5 cases, or 26.3%.

In 2 cases the bile was cultured aerobically and anaerobically and gave a positive culture in both instances, the organism being a gram-positive diplococcus in both.

Culture of the portal vein blood from 5 dogs gave 3 negative cultures and 2 positive cultures. Both positive cultures showed a gram-positive diplococcus.

The culture of the stomach of the 4 puppies gave positive cultures in 100%, and smear from the culture showed the appearance of a gram-negative, spore-bearing bacillus in each case. In 1 of the puppies, long hair was found in the stomach. As the puppy's hair was very short, it was definitely proved that the entrance of hair into the puppy's stomach was from the mother, obtained by the puppy while nursing at the mother's breast. Culture of the liver in the 4 puppies gave positive cultures in only 2 instances. The liver from 1 puppy showed a gram-positive diplococcus and the liver from the second puppy showed a gram-positive bacillus.

From the high percentage of positive cultures and the demonstration of the gram-negative, spore-bearing bacillus in the stomach and duodenum of the normal adult dogs and the stomach of the

puppies, it was evident that there was a constant source of supply of these organisms. Therefore, the following investigations were undertaken. The hair from 5 normal adult dogs and from 4 puppies was cultured aerobically and anaerobically in digest meat broth. Positive cultures were obtained in each case. The gram-negative, spore-bearing bacillus so frequently found in the liver was demonstrated in each culture. Scrapings obtained from the breasts of the nursing mother were cultured, and the same spore-bearing, gram-negative bacillus, as described above, was found.

We believe the source of the organisms found in normal, healthy dogs' liver is obtained from the hair of the animals, the organism gaining entrance into the stomach by way of the mouth, resulting from licking of the body, passing into the duodenum and most probably up through the common duct into the liver. In only 1 case was an attempt made to identify the organisms found in the liver. This was a large, gram-positive organism, and cultural characteristics proved this organism to be the *B. welchii.*

5261

A Method for Determining Basal Metabolism of Fishes.

MAZIE ADKINS. (Introduced by E. S. Hathaway.)

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Various methods used by other workers in metabolism experiments with fishes were considered inapplicable to studies in which physiological experimentation was to be extended over a considerable period of time. The constant flow method used by Hall[1] was modified for the present study. Hall measured the metabolism of many fishes over short periods of time. The present method is adapted for more detailed studies and provides for greater care of the experimental animals. Although Hall employed urethane to inhibit the movement of the fishes, Wieland (1915) states that the use of urethane causes the CO_2 threshold to be raised in the animals thus treated, and Winterstein (1914) concludes that O_2 consumption is decreased with the administration of urethane. Injection with urethane would have to be repeated after a 2-hour period, with consequent mechanical injury and metabolic disturbance to the fish; therefore Hall's method of controlling movement would not be applicable to protracted studies.

[1] Hall, F. G., *Am. J. Physiol.*, 1928, **88**, 212.