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BIOCHEMICAL FOSSILS¹

By Professor DENIS L. FOX

SCRIPPS INSTITUTION OF OCEANOGRAPHY

RECENT studies in this laboratory, to be reported in some detail elsewhere, have been concerned with preliminary qualitative and quantitative examinations of carotenoid pigments encountered in marine sediments of hundreds or thousands of years' standing. The preservation of a class of ordinarily highly labile compounds over vast ages of time is less surprising when one considers the special conditions which prevail in the buried strata of the ocean floor, *i.e.*, lack of free oxygen, absence of light and perpetual temperatures of nearly 0° C. The added facts that carotenoids as a class are absorbed in the digestive tract of most animals only at low levels of efficiency, and that they are relatively refractory toward non-oxidative biochemical changes would still further favor their gradual accumulation in the ocean floor.

¹ Contributions from the Scripps Institution of Oceanography, New Series No. 232.

Pigmentary compounds of the plant and animal porphyrin series have been encountered in petroleum, coal and shale oils.^{2,3} Similarly, other chlorophyll derivatives, accompanied by carotenoids, and sometimes also in association with fluorescent pigments common to petroleum, occur in long and deeply buried marine sediments.^{4,5} These ancient biochromic compounds, together with other oil-soluble substances in whose presence they occur, may be regarded as diagnostic features in the search for biochemical processes in the origins of petroleum and allied natural deposits.

Carotenoids have been reported in moor soils⁶ and

² R. Lemberg, *Ann. Rev. Biochem.*, 7: 424, 1938.

³ A. Treibs, *Ann. d. Chem.*, 510: 42, 1934.

⁴ D. L. Fox, *Proc. Nat. Acad. Sci.*, 23: 295, 1936.

⁵ D. L. Fox and L. J. Anderson, *Proc. Nat. Acad. Sci.*, 27: 333, 1941. (N.B. The published data are in error (p. 335) by a misplaced decimal, thus appearing as 6.0, instead of the correct value of 0.6 mg per cent.)

⁶ O. Baudisch and H. v. Euler, *Arkiv. Kemi Mineral Geol.*, 11A, No. 21, 1934.

field examination. At this time no hamsters were available and urine specimens prepared as described above failed to infect young guinea pigs.

L. canicola Strain "B" was also isolated after the intraperitoneal injection of young hamsters with urine sediment from a dog ill with leptospirosis. This dog of the Boxer type apparently became ill on June 15, 1943. The owner, who became ill and was hospitalized on July 25th, admitted having removed soiled newspapers from the dog's quarters on July 4th. The medical officer in charge of the patient reported that her serum developed an agglutination titer of 1-1,000 for *L. canicola* and a cross agglutination titer of 1-10 for *L. icterohaemorrhagiae*.

On August 10th serum obtained from the dog gave a positive agglutination reaction for *L. canicola* in a dilution of 1-1,000 and a cross agglutination for *L. icterohaemorrhagiae* in a dilution of 1-10. At the same time a urine specimen was obtained by catheterizing the dog. The resuspended sediment prepared as previously described was injected into four young hamsters and four young guinea pigs. On August 22 one of the hamsters died and from its kidneys and liver *L. canicola* was cultured in Fletcher's broth and leptospira demonstrated on tissue section.

ISOLATION OF *L. ICTERHAEMORRHAGIAE* FROM A DOG

On May 26, 1943, a Boston terrier was brought to the dispensary presenting symptoms of leptospirosis. A sample of venous blood was obtained and injected intraperitoneally into two young hamsters which died 6 and 7 days after injection. (From this time attempts to infect guinea pigs was abandoned because of our inability to establish an infection, whereas infections were regularly produced in the hamster.)

Leptospira were observed by the dark-field examination of fresh kidney and liver material, in stained sections, and were cultured in Fletcher's broth.

On May 8th a urine specimen, prepared as previously described, was obtained from this dog and injected into four hamsters, three of which died within 2 to 3 days from bacterial infection. The fourth hamster died of leptospirosis eight days after injection. Marked icterus was evident at the time of death. Leptospira were observed by dark-field examination, in tissue sections, and cultured in Fletcher's broth. Typing of the recovered organism from the blood and urine proved it to be *L. icterohaemorrhagiae*.

This dog had a history of having caught rats in a chicken house on the owner's property. The symptoms manifested in this animal were quite mild in comparison with those in the previously mentioned two dogs ill with *L. canicola* infection. This is the first isolation of *L. icterohaemorrhagiae* from a dog in the United States, previous diagnoses of infection with

this species of leptospira having been based on serologic findings.

Differential diagnosis of *L. canicola* and *L. icterohaemorrhagiae* infections may be achieved by injecting material from the suspect into both young guinea pigs and young hamsters, since only the hamster will succumb to infection with *L. canicola* while *L. icterohaemorrhagiae* infections terminate fatally in both species.

SUMMARY

There is evidence that the golden hamster (*Cricetus auratus*) is the animal of choice for the isolation of leptospirae, especially of the *Leptospira canicola* type since young guinea pigs and mice are resistant to infection and rats are entirely refractory. Both the classical strain, *L. icterohaemorrhagiae* and *L. canicola* on isolation have been found to produce a fatal infection in hamsters.

The present report deals with the isolation of *L. canicola* in two instances by the injection intraperitoneally into hamsters of urine obtained from dogs ill with suspected leptospirosis. Each of the dogs was apparently the source of infection for a human case of *Canicola leptospirosis*.

The classical strain *L. icterohaemorrhagiae* was isolated from the dog for the first time in the United States. In this instance the organism was isolated by injecting both whole blood and urine from the patient intraperitoneally into young hamsters.

The injection into hamsters of suitable material from patients infected with *L. canicola* and *L. icterohaemorrhagiae* is followed by a fatal leptospirosis in the test animal.

RAYMOND RANDALL,
Colonel, Veterinary Corps
HAROLD K. COOPER,
Captain, Veterinary Corps

ARMY VETERINARY SCHOOL,
MEDICAL DEPARTMENT PROFESSIONAL
SERVICE SCHOOLS,
ARMY MEDICAL CENTER,
WASHINGTON, D. C.

BOOKS RECEIVED

- ALEXANDER, JEROME. *Colloid Chemistry Theoretical and Applied*. Vol. 5. Illustrated. Pp. vi + 1256. Reinhold Publishing Corporation. \$20.00.
BRODIE, BERNARD. *A Guide to Naval Strategy*. Pp. xii + 314. Princeton University Press. \$2.75.
ENGARD, CHARLES J. *Organogenesis in Rubus*. Illustrated. Pp. xvi + 234. University of Hawaii.
FIESER, LOUIS F. and MARY FIESER. *Organic Chemistry*. Illustrated. Pp. xii + 1091. D. C. Heath and Company. \$8.00, Trade editions, \$6.00, College editions.
HOWELL, A. BRAZIER. *Speed in Animals*. Pp. xii + 270. The University of Chicago Press. \$4.00.
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