

wave-lengths as compared with the natural mixtures of chlorophyll *a* and *b*.<sup>7</sup> The three bands are at 437, 470 and 675 m $\mu$ , while those for chlorophyll are at 420, 465 and 660 m $\mu$ . The 420 and 660 m $\mu$  maxima of chlorophyll have about the same height, while for phyllochlorin solutions the 437m $\mu$  maximum is always 60 per cent. higher than the 674 band. This suggests the presence of carotenoids associated with phyllochlorin, such as French<sup>8</sup> found for the chromoprotein solutions from photosynthetic purple bacteria.

Boiling a neutral digitalin extract shifts the red absorption band towards the shorter wave-lengths. When a solution is made strongly acid or weakly acid and boiled, the solutions turn yellow, corresponding to the formation of phaeophytins. A digitalin extract saturated with solid ammonium sulfate precipitates the phyllochlorin only after several days, but when boiled forms a bright green viscous mess. No pigment is lost on prolonged dialysis (about two weeks) of a digitalin extract, and only a part of the pigment precipitates. This precipitate, separated by centrifuging, does not readily redissolve in digitalin solution. The pigment which remains in solution is now readily precipitated with high concentrations of ammonium sulfate. Such precipitates are easily redissolved in digitalin solution but not in water. It is likely that the solvent action of the digitalin and the bile salts is due to the formation of coordination compounds which are not broken up even on prolonged dialysis. Phyllochlorin is precipitated and the chlorophyll extracted by strong alcohol, methyl alcohol or acetone but not by petroleum ether in agreement with the effects of these solvents on the leaf. Phyllochlorin solutions show a positive Biuret reaction.

In agreement with observations of the green leaf, phyllochlorin solutions show little or no red fluorescence when irradiated with blue light (436 m $\mu$ ). This is in contrast with the strong red fluorescence of alcoholic chlorophyll solutions. Phyllochlorin solutions are quite stable to visible light.

The behavior of phyllochlorin solutions in strong centrifugal fields is being investigated in collaboration with Dr. E. G. Pickels<sup>9</sup> using an air-driven ultracentrifuge.<sup>10</sup> Preliminary studies show that the phyllochlorin when subjected to a force of 160,000 gravity can be sedimented completely through a 10 mm column of the liquid medium within three hours, leaving no color in the supernatant fluid. Our best preparation

showed two sedimentation boundaries which correspond to particles of high molecular weight, *i.e.*, above 70,000. The two boundaries retained their identity with respect to their sedimentation rates when studied by the light absorption method in the red and blue regions corresponding to the absorption maxima of phyllochlorin in the visible, and in the ultra-violet region characteristically absorbed by proteins. One boundary sedimented almost twice as fast as the other; these more rapidly moving and presumably heavier particles showed a greater total absorption in each of the two regions of the visible spectrum than did the smaller particles.

It is tempting to assume that these two proteins correspond with phyllochlorins *a* and *b*. The similarity of sedimentation properties throughout the spectrum indicates that the additional blue absorption is characteristic of the phyllochlorins and not of some other component.

It now appears that the classical organic chemical studies of the chlorophylls and carotenoids were concerned with the prosthetic groups of extremely complex specific catalysts, perhaps analogous to the hemoglobins and enzymes such as cytochrome, catalase and the yellow respiratory enzyme. Presumably there are many additional components concerned in photosynthesis, since phyllochlorin does not carry on photosynthesis *in vitro*.

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## OVINE AND BOVINE LISTERELLOSIS IN ILLINOIS

THE pathogenesis of organisms of the genus *Listerella* and their possible etiologic significance in rodent septicemia have been reported in England<sup>1</sup> and South Africa,<sup>2</sup> while in New Zealand<sup>3</sup> sheep suffering from an encephalitic syndrome, designated "circling disease," has been associated with or attributed to *Listerella* infection. In the United States, *Listerella* has been isolated from cattle,<sup>4</sup> sheep<sup>5</sup> and man,<sup>6,7</sup> displaying symptoms of encephalitis, and from chickens<sup>8</sup> that disclosed lesions of necrotic myocarditis. So far as

<sup>1</sup> E. G. D. Murray, R. A. Webb and M. B. R. Swann, *Jour. Path. and Bact.*, 29: 407, 1926.

<sup>2</sup> J. H. H. Pirie, *So. African Inst. Med. Res.*, 3: 163, 1927. Cited by Gill.

<sup>3</sup> Dudley A. Gill, *Vet. Jour.*, 87: 60, 1931 and 89: 258, 1933; *Australian Vet. Jour.*, 13: 46, 1937.

<sup>4</sup> F. S. Jones and R. B. Little, *Arch. of Path.*, 18: 580, 1934.

<sup>5</sup> Erwin Jungherr, *Jour. A. V. M. A.*, 91: 73, 1937.

<sup>6</sup> Caspar G. Burn, *Jour. Bact.*, 30: 573, 1935; *Am. Jour. Path.*, 12: 341, 1936.

<sup>7</sup> E. W. Schultz, M. C. Terry, A. T. Brice and L. P. Gebhardt, *Proc. Soc. Exp. Biol. and Med.*, 31: 1021, 1934. Cited by Burn.

<sup>8</sup> C. V. Seastone, *Jour. Exp. Med.*, 62: 203, 1935.

<sup>7</sup> *c.f.* E. Rabinowitch and J. Weiss, *Proc. Roy. Soc. London, A*, 162: 251, 1937.

<sup>8</sup> C. S. French, Abstract in the Proceedings of the American Society of Biological Chemists, Baltimore meeting, March 30-April 2, 1938.

<sup>9</sup> Of the Laboratories of the International Health Division, The Rockefeller Foundation, New York.

<sup>10</sup> J. H. Bauer and E. G. Pickels, *Jour. Exp. Med.*, 65: 565, 1937.

we are aware, listerellosis in sheep and cattle showing an encephalitic and/or an encephalomyelitic syndrome has not been reported in central United States. However, Doyle<sup>9</sup> described an idiopathic encephalitis of sheep in Indiana accompanied by gross and microscopic changes in the brain analogous to ovine *Listerella* infection encountered in Illinois during the winter of 1937-38.

In this paper reference is made to two outbreaks of ovine and one outbreak of bovine encephalitis and/or encephalomyelitis associated with *Listerella*. The first outbreak coming to the attention of the Illinois Agricultural Experiment Station occurred in a group of 250 feeder lambs that had been purchased at a central market. Lambs of this type for fattening generally originate in western grazing states, but the origin and history of the animals comprising this shipment were not determined. Approximately six weeks following arrival of the lambs on a farm in DeWitt County, Ill., symptoms of illness developed. The symptoms consisted of depression, weakness, incoordination, fever, walking in circles, pushing against objects with the head, anorexia and progressive paralysis, terminating in coma and death. Approximately 30 lambs died or became moribund and were destroyed and a few mildly affected lambs apparently made a complete recovery. An ophthalmia of transitory nature was noted in some of the mildly affected lambs. The same lesion was also observed at autopsy of fatally affected lambs.

Five of the typically affected lambs from this flock came to autopsy. Each yielded *Listerella*-like organisms from the brain stem on liver agar plates and/or in tubes of meat mash media incubated at 37.5° C. Bacteriologically sterile brain tissue filtrates (Berkefeld N) proved negative to demonstrable virus upon intracerebral inoculation of rabbits, guinea pigs and chickens.

The second outbreak occurred in a flock of 100 breeding ewes in DeWitt County, but so far as determined, had no connection with the first outbreak. A small number of the breeding ewes in this flock displayed symptoms involving the central nervous system with a mortality of six ewes. One of the clinically affected animals was submitted for observation and autopsy. An organism possessing characters of the genus *Listerella* was isolated from the brain stem.

A third outbreak showing an encephalitic and/or encephalomyelitic syndrome was observed in a group of 60 yearling feeder steers in Piatt County, Ill. The principal symptoms were glassy, dazed expression of eyes, partial paralysis of mandible, elevation of the head and salivation. Affected animals became prostrate and remained comatose for three to four days

before death. *Listerella*-like organisms were isolated from the brain stem of two fatally affected steers.

Postmortem examination of naturally affected lambs and ewes in two outbreaks did not reveal any marked gross pathologic changes in the internal organs. In some of the lambs the cervical and visceral lymph nodes were enlarged and slightly edematous. There was an increased amount of slightly cloudy cerebrospinal fluid with slight congestion of the meninges. In one case gross lesions suggestive of a localized meningitis were noted. The blood picture of five of the natural ovine cases did not show any significant deviation from the normal.

An examination of stained sections from the brain of naturally affected lambs and cattle showed polymorphonuclear and mononuclear foci in the stem and in the white matter of the cerebrum and cerebellum, together with perivascular cuffing with mononuclear cells and a mononuclear meningitis.

The pathogenic properties of *Listerella* strain isolated from one outbreak in sheep have been established by artificial exposure of healthy lambs, calves, chickens, guinea pigs, rabbits and rats, while cultural and biochemical properties of the strains from the three outbreaks described herein conform to the genus *Listerella*. It appears that three natural outbreaks (two in sheep and one in cattle) in Illinois, accompanied by symptoms of encephalitis and/or encephalomyelitis, were associated with *Listerella* infection.<sup>10</sup>

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#### THE EFFECT OF NUCLEOPHOSPHATASE ON "NATIVE" AND DEPOLYMERIZED THYMONUCLEIC ACID

It has been definitely established that desoxyribonucleic acids differ in their molecular weight, depending upon their method of preparation. The substance prepared by E. Hammarsten<sup>1</sup> seems to be the un-

<sup>10</sup> After dictating the above report on the occurrence of listerellosis in cattle and sheep in Illinois, attention was called to an article on "Listerella Infection in Fowls in East Anglia" by Dr. J. Stuart Paterson, Institute of Animal Pathology, Cambridge University, published in *Veterinary Record* 49: 49, 1937, with reference to spontaneous *Listerella* in four separate groups of fowls, as follows:

a. Two adult fowls were involved, and in both cases death occurred suddenly.

b. One hundred and twenty out of 200 Leghorn pullets died during a period of three months. In addition to the presence of organisms of the *Listerella* group, *B. pullorum* was recovered from some of the pullets and there was also a heavy infestation of tapeworms (*Davainea proglottina*).

c. Four hundred pullets and 24 stock cockerels comprised the affected unit. The losses were 190 pullets and one cockerel. In several of the pullets from which *Listerella* were recovered, lesions of fowl paralysis (neurolymphomatosis) were also present.

d. Eight out of 24 young poultry died.

<sup>1</sup> E. Hammarsten, *Biochem. Zeits.*, 144: 383, 1924.

<sup>9</sup> L. P. Doyle, *Jour. A. V. M. A.*, 81: 118, 1932.