THE

JOURNAL OF

COMPARATIVE PATHOLOGY

AND

THERAPEUTICS.

Vol. XXVIII.—No. 3. SEPTEMBER 30, 1915. PRICE 2s. 6d.

MONASCUS PURPUREUS (WENT) NOT A CAUSATIVE FACTOR IN FORAGE POISONING.

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MANY of the common moulds have been indirectly incriminated as a causative factor in those diseases of live-stock which in all probability originate from contaminated food. The many names given to the disease popularly known as forage poisoning have made the nomenclature somewhat misleading. Until something definite has been determined it is undoubtedly wise to consider all diseases of this nature as one and the same. Since the food always appears to be involved, we would suggest "forage poisoning" as a more appropriate designation than "blind staggers," "cerebritis," "meningitis," or any other name. The popular conception among bacteriologists and veterinarians is that this disease results in a disturbance of the central nervous system, particularly the meninges; hence the term "cerebro-spinal meningitis." It is altogether possible that this conception is erroneous. The only real evidence of the correctness of this view is the clinical symptoms manifested; the congestion of the meninges quite uniformly observed on *post-mortem* examination could easily be accounted for as the result of diseased processes in other organs of the body.

No attempt will be made in this paper to review the entire literature dealing with forage poisoning, but only that which has a bearing on the significance of moulds, particularly monascus purpureus (Went),[1] inasmuch as this organism has been looked upon with suspicion in other outbreaks.

[1] Identified independently by Professor F. T. M'Farland of the University.

Most of the investigators of this disease have attempted the incrimination of one or more of the common moulds as the etiological factor of this disease. Thus Mayo (1) attributed forage poisoning to the mould aspergillus glaucus, which occurred abundantly on corn suspected of having been involved in one outbreak. This corn was found to produce the disease when fed to experimental animals. No feeding experiments with pure cultures of aspergillus were reported. Haslam (2) also looked upon moulds as possessing pathogenic powers. He isolated three different species, viz., aspergillus flavus, aspergillus niger, and mucor rhizapodoformis. These were fed to rabbits without results. The mucor would kill rabbits in three days when injected intravenously. From silage responsible for the death of several horses, Buchanan (3), of Iowa, isolated and studied morphologically monascus purpureus (Went). Healy and Garman (4) isolated this organism from a corn thought to be responsible for an outbreak in Kentucky, but when fed to guinea-pigs no injurious effects were noted. In several outbreaks it has been suggested that monascus purpureus (Went) might be of etiological significance, and in a measure this fact has been half way accepted without convincing evidence. Previous to this time no experimental work to prove or disprove this idea has been reported.

Opportunity for the study of forage poisoning was afforded by a typical outbreak of the disease in this vicinity. In experiments conducted by feeding the various available foods to horses on one farm we obtained conclusive evidence that oat-hay, which constituted one of the foods, was the exciting cause of the disease in horses and mules (5). We have since been able to reproduce the disease at will by feeding this material, either the shelled grain or the grain-free straw. In laboratory studies of the material several organisms have been isolated for study. Among them monascus purpureus (Went) has been studied extensively, and, because of the suggestive position accorded it in the forage poisoning problem previous to this time, it was used as the basis of the experiments herein presented.

The theory of toxin or poison-producing fungi as a cause of this disease is supported by the history of all forage poisoning outbreaks, and, while the plausibility nearly reaches the point of conviction, the incrimination of fungi on purely circumstantial evidence is unwarranted. In the history of medical science few diseases other than local affections have been proven to be caused by fungi of the class of hyphomycetes. Only under exceptional conditions do these fungi invade the internal organs not accessible from without, as, for example, the spleen. The living tissues of the animal body do not afford a suitable habitat for organisms of this nature; the oxygen and temperature requirements of the hyphomycetes in general militate against such an invasion. According to the investigations of Lichtheim and others, the conidia of aspergillus fumigatus will grow in animal tissues when introduced into the blood stream, but no new conidia are formed, progressive infection being therefore prevented, and the diseased processes limited to the inoculated area. A conception of disease of the nature of forage poisoning being produced by fungi of whatever nature must of

necessity assume the products of their metabolism to be the exciting cause, rather than any action necessitated by the presence of the organismal body in the circulation or organs of the affected animal. If this be a tenable point of view, we must ascribe to fungi the power to produce true soluble toxins. The only other possible explanation is that substances which simulate ptomaines are produced in plants by fungi.

The nature of the metabolic products of fungi, the hyphomycetes in particular, is still uninvestigated, at least in so far as their physiological action on animals is concerned. Warthin (6) cites Ceni as authority for the statement that aspergillus fumigatus under certain conditions produces poisons, one of which can be extracted with alcohol, and which when injected into rabbits will produce tetanic convulsions. To aspergillus niger has also been ascribed the power to produce certain amounts of oxalic acid. These points, it is quite evident, demand confirmation. The aspergillus fumigatus, above referred to, the recognised cause of a respiratory disease of birds, has always been supposed to produce its pathological effects by the inflammatory reaction excited, mechanically, by the proliferation of the mycelia in the tissues. This is also the case in those local affections of man and animals caused by fungi of which our knowledge is more or less complete. Thrush, for example, caused by oidium albicans, has been considered as an inflammatory condition due to the irritating presence of the etiological factor. To further support this contention, we wish to call attention to the fact that monascus purpureus has not been isolated from the body tissues of animals suffering from forage poisoning.

In the work which we desire to present, monascus purpureus has been fed, and the filtered products have been injected intravenously. The feeding experiments presented below involve work on seven horses, and on guinea-pigs, white rats, rabbits, chickens, and a hog.

The material was grown on oat infusion, and was not used until a very luxuriant growth had been obtained. This material was consumed by the small experimental animals in drinking water with no ill effects. The experiments on the larger animals were as follows:—

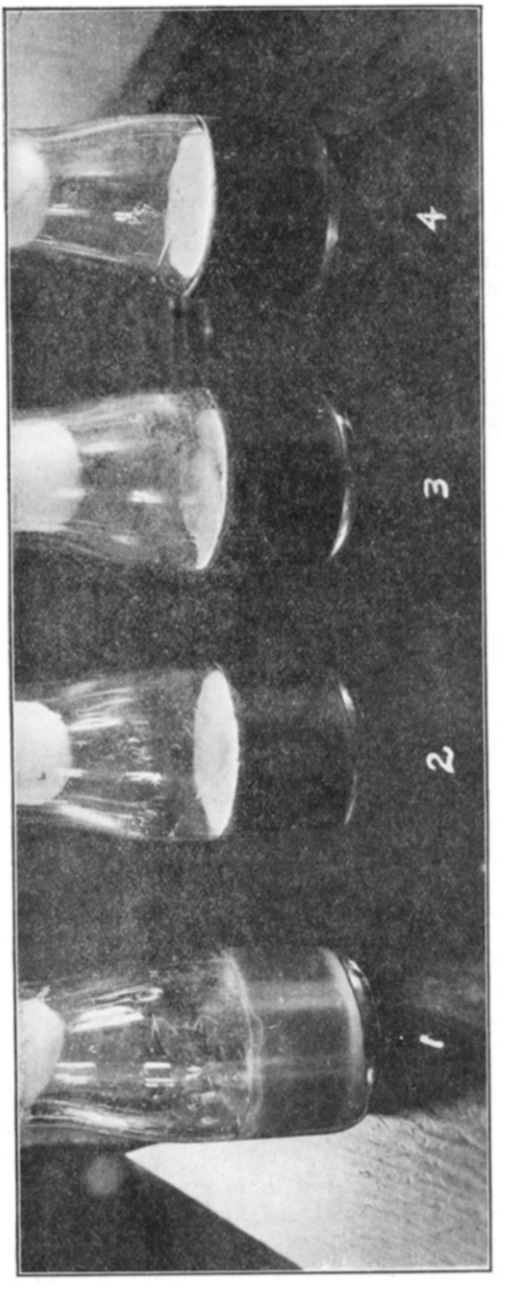
1. Horse No. 1. 100 cc. of monascus purpureus was given on corn-meal daily for thirty-two consecutive days. Cultures were of varying ages. The youngest was of seven days' growth, while the oldest was of twenty-eight days' growth. We feel that by feeding cultures of different ages we have furnished the material to the experimental animal in all possible stages of its growth on artificial media, and in case deleterious products are evolved at certain stages of the growth of monascus they would have been produced during the time allowed. No ill effects were noted.

2. Horse No. 2. 100 cc. of monascus purpureus given daily on corn-meal for nine consecutive days. No ill effects.

3. Horse No. 3. Monascus supplied daily in drinking water for eleven consecutive days. The first culture given was of nine days' growth. The remainder varied from twelve to twenty days. No ill effects.

4. Horse No. 4. Received 100 cc. of monascus culture on feed each day for eleven consecutive days. No ill effects.

5. Horse No. 5. Received 100 cc. of monascus culture on feed daily for twenty-five consecutive days. No ill effects.



Monascus purpureus (Went).

1. Six day culture. 2. Fourteen day culture. 3. Twenty-one day culture. 4. Twenty-six day culture.

6. Horse No. 6. Received 100 cc. of monascus culture daily on feed. Duration of feeding fourteen consecutive days. No result.

7. Horse No. 7. Received as an exclusive ration oats which had

been sterilised and inoculated with monascus. After incubating thirty days a very heavy growth resulted. This was fed for fourteen consecutive days. No ill effects.

8. Hog. 100 cc. of culture daily for twelve consecutive days in feed. No results.

In view of the fact that horses fed with the oat-hay from which this monascus was isolated developed symptoms of the disease in eight to fourteen days, it seems to us that the experiments presented can be accepted as evidence of the non-pathogenicity of monascus purpureus, as isolated from these oats. The possibility exists, however, that laboratory methods of cultivating this organism will not answer the purpose demanded for the production of toxic substances *in vitro*. On the other hand, it is possible that the poisonous substances in the oats were produced in the plant before the grain reached maturity.

Experiments in which the organism-free liquid from cultures of the monascus was injected intravenously were also conducted. In case of the liberation of poisons by monascus purpureus during its growth on cultural media, one could expect results from the injection of this material. Four horses were treated with infusion upon which the mould had grown. The organism was removed by filtration through filter paper, after which the liquid was filtered through Kitasato porcelain filters. Each animal received 100 cc. of the sterile filtrate intravenously daily for four days without effect. Sterile filtrates were also injected into sheep, rabbits, and guinea-pigs without results.

Other Moulds.

One horse was fed exclusively for thirty days on mouldy oats. These oats were contaminated with a number of moulds, such as rizopus nigracans, aspergillus niger, and penicellium sp. The animal never evidenced symptoms of the disease in question, and gained in body weight.

Discussion.

While the experiments herein presented deal with but one outbreak of so-called forage poisoning, and for the most part with but one fungus, we feel that they settle to a degree the question of the pathogenicity of monascus purpureus as grown under artificial conditions in the laboratory. The fact that forage poisoning has been said to have been caused by the feeding of foods other than oats has no bearing on the question at hand. In our opinion the kind of food has no relation to the disease; in other words, the food is but a fomite by which the causative substances gain entrance to the animal body. We would prefer to take the position advanced previously in this paper, that in case fungi are involved the poisonous substance might be evolved by the fungus during the growth of the host plant and stored in the plant.

Under laboratory conditions we have been unsuccessful in attempts to detect the production of toxic substances by monascus purpureus. If the monascus purpureus were capable of producing true soluble toxins it is reasonable to suppose that they would have been produced under the conditions of our experiments, since little difficulty

is experienced in producing diphtheria or tetanus toxin *in vitro*. Further evidence of the non-pathogenicity of monascus purpureus was called to our attention by Professor Buchanan, of Iowa, who cited Lafar as stating that rice reddened by monacus purpureus is considered a delicacy by the Asiatic people.

Conclusions.

We feel that the experiments herein presented are suggestive of the following conclusions:—

Monascus purpureus (Went) grown under laboratory conditions had no etiological significance in this outbreak of forage poisoning, since feeding large quantities over sufficient lengths of time did not produce the disease. We desire to present the evidence of the foregoing experiments as suggestive of the non-pathogenicity of monascus purpureus (Went) when fed in large quantities, and when the products of its metabolism are injected intravenously.

Monascus purpureus isolated from oats which had undoubtedly given rise to forage poisoning in horses and mules did not produce soluble or extracellular toxins *in vitro* on the cultural media employed, as shown by absence of clinical symptoms in the experimental animals.

LITERATURE.

(1) Mayo: Bul 24, Kas. Expt. Station.
(2) Haslam: Bul. 173, Kas. Expt. Station.
(3) Buchanan: "Mycologia," Vol. II., No. 3, 1910, p. 99.
(4) Healy and Garman: Biennial Report, Ky. Expt. Station, 1911-13.
(5) Graham: "Breeder's Gazette," 13th May 1915.
(6) Warthin: "Zeigler's Pathology," A. S. Warthin (Ed.), p. 682.

ON A FŒTAL SAC FOUND ATTACHED TO THE GASTRO-SPLENIC OMENTUM OF A RABBIT.

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ALTHOUGH evidence has been accumulating during recent years which strongly supports the view held by Bland Sutton and others that the so-called cases of abdominal pregnancy are due to uterine rupture with subsequent extrusion of the fœtus, still an account of a new case may not be entirely superfluous.

In 1893 a doe rabbit, opened for class purposes by Professor Philip J. White, was found to contain a large tumour attached by a pedicle to the gastro-splenic omentum, but otherwise free in the abdominal cavity. The interest of the condition was recognised, the specimen was preserved for future examination, and it now forms the subject of this paper.

The sac is sketched in fig. 1; it might perhaps be described as stomach-shaped, and showed a greater and lesser curvature. The stalk was attached towards one end of the lesser curvature, and was about 3·5 cm. long. The sac itself was about 6·5 cm. long, 4·5 cm. deep, 3·5 cm. thick at its greatest dimensions.