THE INHIBITORY EFFECT OF ACRIDINE ON THE SPOROGONY OF A COCCIDIUM (EIMERIA STIEDÆ).

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The common coccidium of the rabbit is transmitted from animal to animal through the medium of an encysted stage which is deposited with the feces and then undergoes a period of ripening during which the enclosing membrane remains intact. The cyst is then capable of infesting fresh animals and probably of reinfesting its own host. The ripening process consists in the development within the oocyst of four sporoblasts, then of four spores from these, and finally of eight sporozoites by the internal division of each spore into two.

The oocyst is characterized by a heavy, double contoured wall which appears to be a very effective protection for the protoplasmic structures within. The development proceeds in the presence of a reasonable amount of moisture and at ordinary summer temperatures under a wide range of surrounding conditions. Heavy cultures of the yeasts, molds, and bacteria of rabbit feces may develop about the cysts without interfering in the slightest with their ripening. Free oxygen is said to be essential to the process. It is also said that considerable concentrations of the common disinfectants are withstood.

The coccidium disease of the rabbit is a serious one for those who attempt to raise this animal, as under otherwise good conditions it often causes a very high mortality among the young. The disease is also the prototype of diseases in cattle and poultry which frequently occasion considerable losses. Human beings are sometimes affected. In the rabbit the disease consists of an infestation of the epithelial cells of the small intestine and the bile ducts in the liver. In other

animals the liver is not invaded and in the rabbit it is by no means always involved. The liver has been involved in some human cases. Spread of the disease occurs within the intestinal tract through the medium of merozoites which are for a longer or shorter time free in the contents. It would seem not unlikely that the parasites, in this stage at least, might be more than ordinarily accessible to the action of drugs. However, when one considers what is known of coccidial disease from the point of view of treatment, the available information is meager in the extreme. In the fatal cases the oocysts of the parasite are present in the feces in numbers, at times it is stated in enormous numbers. The more severe stages of the disease are apt to be marked by a diarrhea and in cattle this is frequently bloody.

The length of time occupied by the earlier stages of the disease is not known. Nor is it known whether the number of oocysts in the feces varies in such a way as to give any clue to the progress of the disease in the host. There is no other test which may be applied to control progress.

In the light of this general situation it was thought that further study might be of eventual help and, as one step, that it would be of interest to see whether certain chemical compounds which are known to act against other protozoa, either in the test-tube or therapeutically, would have an influence on the ripening process of the oocysts of the rabbit's coccidium. Observations on this point seem not to have been reported previously.

Methods.—Feces or the contents of the large intestine were rubbed in water to make a heavy suspension. This was, when necessary, strained through a layer of fine gauze to remove the larger fragments of vegetable fiber. The cloudy suspension was mixed with an equal volume of a saturated solution of cane-sugar, shaken well, and centrifuged. (International Centrifuge, Size 2, Type B, rheostat set at 16, time 15 minutes.) The oocysts are thus concentrated at the top of the tube and were removed by first stirring the top centimeter of fluid and then dipping into it a large wire loop of the type used for transferring bacterial cultures. Owing to peculiar adhesive qualities of the cysts they tend to come away with the first few loopfuls. It was found advantageous to prepare several small tubes rather than one large one. With material containing cysts in abundance it is possible to dip ten or fifteen times in each tube and get many cysts each time. After this they become noticeably less abundant.

Agar was dissolved in water to make a 2 per cent gel. This was tubed, 9 cc. per tube, and sterilized. For use this was poured into Petri dishes, a loopful or more of the suspension was placed on the surface at a marked spot after solidification. This preparation was kept in a warm room (between 70° and 80°F.), in the dark, during the observation period. The plates were opened daily and a drop or two of water put on the planted spot. The method of concentration described is adapted from that proposed by Sheather[1] for eggs of parasites in feces. The method of culture is essentially that used previously in this laboratory by Smith and Graybill.[2]

The tests of action of the various substances were made by adding them, before pouring the plates, to the melted agar in appropriate quantities together with sufficient water to make 10 cc. in the tube.

Observations of the progress of the cultures was made with the low powers of the microscope through a drop of water. This was checked at critical points by dropping a cover-slip on the culture and observing with higher powers. Cultures under the cover-slip seemed to proceed with much less regularity, probably due to unevenness in the oxygen supply.

The following substances were tested by this method. The figure in parenthesis following the name is the denominator of the dilution fraction of the greatest concentration employed. Thus quinine hydrochloride (100) means that the 1/100 dilution of this substance was observed as a maximum concentration.

1. Compounds known to be active against other protozoa: Quinine HCl (100); tartar emetic (100); *p*-aminophenylarsenoxide (100); emetine (1,000); acridine HCl (10,000); proflavine (1,000); methylene blue (10,000); trypan red (2,000); trypan blue (10,000).

2. Compounds related to acridine:[3] Acridine orange (1,000); acridine red (1,000), phosphine N. Bad. (1,000); chrysaniline Kahl. (1,000); phosphine 3 R (500); phosphine G. (1,000); acridine yellow (1,000); cyanotrypaflavine (1,000); 9-methylacridinium methochloride (1,000); leuco-cyano trypaflavine (1,000); 3-6-diamino-9-phenylacridine (1,000); 3-amino-6-hydroxyacridine dihydrochloride (1,000); 3-6-diaminoacridine (1,000); 3-aminoacridine (1,000); 2-7-diaminocarbazole (1,000); 2-7-diamino-9-phenylacridine (1,000); diaminonaphthacridine, impure, (1,000); 9-phenylacridine (1,000).

3. Other compounds: Safranine$_w$ (2,000); Nile blue sulfate (10,000); methyl violet 6 B (10,000); malachite green (10,000); neutral red (10,000); eosin (2,000);

[1] Sheather, A. L., *J. Comp. Path. and Therap.*, 1923, xxxvi, 71.

[2] Smith, T., and Graybill, H. W., *J. Exp. Med.*, 1918, xxviii, 89.

[3] I am indebted to Dr. W. A. Jacobs, of The Rockefeller Institute, New York, for the unusual compounds in this group as well as for a helpful interest in the problem.

benzidine (saturated); acetanilide (saturated); indazole (saturated); trimethylamine HCl (100); para-phenylendiamine (100); aniline HCl (100); quinoline yellow (1,000); quinoline hydrochloride platinic salt (1,000); isoquinoline hydrochloride platinic salt (1,000); β-naphthoquinoline HCl (1,000).

Of these compounds only one, acridine hydrochloride, has shown any effect. This substance completely inhibits the ripening of most of the oocysts under certain conditions which may be briefly discussed.

In concentration of 1/10,000 development of nearly all the cysts is entirely inhibited. Concentrations down to and including 1/80,000 act in the same way. In concentrations 1/100,000 to 1/1,000,000 development is still inhibited but in lesser and decreasing degree. The inhibition in this range manifests itself in delay and in slower progress in contrast to the more concentrated range where, with certain exceptions, development is completely prevented. The oocysts on the plates in the concentrated range apparently lie dormant. The nuclear matter undergoes no visible change either of development or degeneration.

There is, however, an exception to and also an interesting limitation on these statements. As an exception it is to be noted that an occasional cyst undergoes its normal development regardless of the concentration of acridine to which it is exposed. The sporoblasts are formed with the controls and the ripening process is completed in the usual way and in the usual time. Furthermore, if the oocysts are allowed to stand in a warm room for 24 hours until sporoblasts have been formed and then are planted in the acridine plates the development proceeds normally to the formation of sporozoites.

The exceptional development of an occasional cyst may be an instance of "fastness" such as has been commonly noticed in chemotherapeutic experiments with trypanosomes.

The fact that after sporoblasts are formed acridine no longer has an influence is interesting indicating as it does an action of the compound very precisely directed at a particular condition of the parasite, a particular stage in the parasite's life cycle.

Ehrlich when developing the results of his chemotherapeutic experiments held as closely as possible to the idea that his compounds acted, either as such or in modified form, directly as germicidal

agents against the parasite. Others since have shown that in some instances the action of the compound must be on the host rather than on the parasite; chemicals may stimulate the body cells of the host to the production of antiparasitic substances, or perhaps to other antiparasitic action. This general question of mechanism has been well reviewed recently by Dale.[4]

This action of acridine on the oocysts of the coccidium in the resting stage, contrasted with its failure to act after sporoblasts are formed, presents a nice example of action specifically directed and directly exerted.

SUMMARY.

The development or ripening of the oocyst of the coccidium of the rabbit is prevented by acridine hydrochloride provided that the cysts are exposed to the action of the chemical before development has started. After sporoblasts are formed acridine does not prevent further development. Many other substances, some of them known to be active against certain protozoan parasites, have no influence on the ripening of the oocysts of the coccidium.

[4] Dale, H. H., *Physiol. Rev.*, 1923, iii, 359.