

The Effect of Poisons on the Neuromotor System of Ichthyophthirius

By RONALD F. MacLENNAN
State College of Washington

Definite coordinated movements and specific behavior patterns have been recognized in the protozoa, particularly the ciliates, since the work of Jennings (1906). The morphological basis of these

behavior patterns was unknown until Sharp (1914) described a centered system of fibrils linking all the cilia into a single morphological unit, the nepromotor system. Taylor (1920) and various later

by slope gradients. The rate of acceleration of both kinds of erosion is rapid on most lands in the Northwest.

3. The problems of accelerated erosion in the Intermountain Zone of the Pacific Northwest are different from those in the North Pacific Zone. Control of erosion is also different in these two divisions. The damage caused by erosion in the Intermountain Zone is mostly direct, while that in the North Pacific Zone is also indirect. In the latter floods are especially destructive.

4. The distribution of erosion by classes on agricultural and grazing land in the Intermountain Zone shows that the larger part of each type is already seriously eroded, only a small proportion is adequately protected and little is entirely ruined, and that much of the land can be retained for its present use if erosion control measures are immediately instituted. Some general control measures are suggested. On forest lands, serious erosion is less extensive but has occurred.

5. It is shown that there is a critical point after which the removal of slightly more cover or continued improper land management greatly accelerates erosion processes. The indirect effect of erosion after this point is reached is often as important as actual soil losses. Reference is made to run-off, ground water supplies, floods, soil accumulations and natural re-vegetation.

6. Restoration of vegetation by natural succession under proper protection is still possible and economical on most of the range and forest lands. If erosion con-

tinues unchecked this cannot be done. There is an apparent balance between the degree of cover depletion, erosion and revegetation. On agricultural land important changes in use and management must be made to protect soil during critical erosion periods.

7. It is probable that geologic norms of erosion are a factor in flood conditions in the North Pacific Zone. Accelerated erosion also occurs. The exact extent of each of these kinds of erosion is not known but more attention must be given to the control of the accelerated phase.

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LITERATURE CITED

- ¹ Lowdermilk, W. C. Acceleration of Erosion above Geologic Norms. National Research Council, Transactions of the American Geophysical Union, 2: 505-509. 1934.
- ² Bennett, H. H., and Chapline, W. R. Soil Erosion a National Menace. U. S. Dept. Agric. Cir. 33. 1928.
- ³ Munns, E. N., W. R. Chapline, R. D. Forbes, L. F. Kellogg, H. G. Meginnis. Watershed and Other Related Influences and a Watershed Protective Program. Senate Document XXI, (5): 304. 1933.
- ⁴ Rockie, W. A. Some Important Effects of the Summer Fallow System of Farming on Soil Erosion in the Pacific Northwest. Northwest Science, VII, (1): 19-22. 1933.
- ⁵ Frissell, S. D., G. L. Fuller, G. W. Musgrave and C. E. Ramser. Glossary of Terms Used in Soil Conservation. U. S. Dept. Agric., Soil Conservation Service. 145 pp. Mimeographed. 1936.

workers severed some of these fibrils by microdissection methods and demonstrated a resultant disorganization which they attributed to the severing of the neuroid fibrils. The natural occurrence of a similar condition was found in the fish-parasite *Ichthyophthirius* (MacLennan 1935) during the reorganization following multiple divisions. The longitudinal somatic fibrils lose all connection with each other and with the controlling centers. The behavior pattern also shows a characteristic change—the ciliate merely rolls around aimlessly instead of proceeding rapidly in a spiral course as is the case when the fibrils are connected. Klein (1934) produced a disorganization both in the neuromotor system and in the behavior pattern of free-living ciliates by exposures to poisons, alkaloids, ultra-violet rays, x-rays, and radium emanation. The purpose of the experiments recorded below was to repeat and to extend some of Klein's work using *Ichthyophthirius*, a ciliate whose cytology is thoroughly known for all stages in the life cycle. The experiments were designed to show whether, in dilute solutions, poisons are specific for the neuromotor system and also to see whether there is any variation in susceptibility in the various parts of the neuromotor system.

METHODS

The following solutions were used: N/20 KCN, ¼% Chlorotone, 1% neutral red, and 1% janus green. This rather miscellaneous group of reagents was selected for the experiments because their action on the ciliate is relatively slow so that the cytoplasmic changes can be studied in detail. A series of dilutions was made with tap water (a medium in which the ciliates can encyst and reproduce freely) and groups of ciliates were placed in each dilution. Thus individual variations in susceptibility were counterbalanced. The use of two "vital" dyes as poisons was suggested by observations on overstained specimens. They produce no abnormalities

when used in sufficiently dilute solutions, but in stronger solutions are definitely deleterious. The most instructive cases were those in which death occurred in not less than 24-28 hours. Shorter and more violent deaths do not allow sufficient time for gauging interference with such processes as redifferentiation. Some series were allowed to proceed undisturbed, in others characteristic specimens were fixed at intervals and impregnated by Klein's silver-line method.

OBSERVATIONS

None of the solutions used was found to be specific for the neuromotor system. The contractile vacuoles show the first signs of abnormality, then the endoplasm, and finally the neuromotor system. The nuclei are extremely resistant and show little or no change even when the pellicle has ruptured and they lie naked in the surrounding medium.

Changes in the contractile vacuole are the most delicate indicators of abnormality. Mild poisoning does not hinder the accumulation of water and the enlargement of the vacuole, but it does hinder and finally stop the discharge of the fluid contents. Systole is initiated by a solution of the surrounding cytoplasm (MacLennan 1932) but in the poisoned individuals, the cytoplasm which is normally in a thin gel during diastole, cannot return to the sol form. As a result, tremendous vacuoles are formed which distend the whole individual and finally discharge internally to dilute the endoplasm. Brinley (1928) and Lepeschkin (1932) working on other aspects of narcotics and poisons also found this initial increase in the viscosity of the cytoplasm. The effect is not specific for the poison used but is found with all the solutions used.

The coordination of the neuromotor system is apparently unaffected during these early stages. Normal behavior, including the feeding reaction, continues so that large amounts of water continue to be

ingested with the food vacuoles. This heightens the effect of the failure of diastole in the contractile vacuoles.

Definite changes in the neuromotor system do occur after a considerable period. First, the oral cilia beat more slowly than the body cilia and finally cease altogether. Then the body cilia lose their coordinated beat and finally become quiescent. When the fibrils are stained according to the silver methods of Klein, it is seen that they have become thin and inconspicuous. This change is difficult to use as a criterion of change because of the amount of variation inherent in methods using silver impregnation. To overcome this difficulty young ciliospores were taken during the period when redifferentiation of the mouth occurs in the hope that young fibrils in the process of formation would be more susceptible and show definite morphological evidence of disturbance. Positive results were attained with chloretone in 1/4-1/8 saturated solution.

The number of oral fibrils were counted in two different series both of which were fixed and impregnated 24 hours after excystment. One series was the normal control, the other had been immersed in 1/8 saturated chloretone for 24-36 hours. The normal controls possessed 5-9 oral fibrils, and 65% had 6. The poisoned individuals had 0-3, 60% had only one. In the same two series the number of longitudinal somatic fibrils on one side were counted as a measure of the changes in the somatic cilia. The number of rows in the normal controls was 19-27, in the poisoned individuals 21-26. Fifty per cent of the controls had 20-22 rows, 60% of the poisoned ones had 21-22 rows. The total number of somatic rows in each ciliate is very nearly twice the numbers given above. The dry silver-line method stains only the outer half-surface, the half-surface attached to the slide is unstained.

The differentiation of the mouth is inhibited by chloretone, the somatic fibrils,

however, were undisturbed. The somatic fibrils undergo most rapid multiplication some time before excystment, while the oral fibrils are differentiated to a large extent after excystment. The conditions of the experiment were such that the inhibiting environment was applied to fully differentiated somatic fibrils and to oral fibrils right in the process of differentiation. Thus a dilution which is insufficient to destroy structures already laid down does inhibit their differentiation. This result can be compared to the critical periods found by Stockard in fish embryos and also to the greater susceptibility in regions of greatest activity, found by Child and his co-workers.

CONCLUSION

Inhibition of differentiation of the neuromotor system can be induced by the use of chloretone. Once the fibrils are laid down they are relatively resistant and only slight changes can be produced before the death of the individual.

LITERATURE CITED

- Brinley, L. 1928. Effects of cyanide on the protoplasm of amoeba. *J. Gen. Phys.* 12: 201.
- Jennings, H. S. 1906. Behavior of the lower organisms.
- Klein, B. M. 1934. Strukturelle und formative Reaktionen des Silberliniensystems. *Ann. Protistol.* 4: 55.
- Lepeschkin, W. W. 1932. Some aspects of the causes of narcosis. *Physiol. Zool.* 5: 479.
- MacLennan, R. F. 1933. The pulsatory cycle of the contractile vacuole. *Univ. Calif. Publ. Zool.* 39: 205.
1935. Dedifferentiation and redifferentiation in *Ichthyophthirius*. I. Neuromotor system. *Arch. f. Protistenk.* 86: 191.
- Sharp, R. G. 1914. *Diplodinium ecaudatum* with an account of its neuromotor apparatus. *Univ. Calif. Publ. Zool.* 13: 43-122.
- Taylor, C. V. 1920. Demonstration of the function of the neuromotor apparatus in *Euplotes* by the method of microdissection. *Ibid.* 19: 403.

Editorial Postscripts

With this issue of N. W. S. the present editor resigns. It has been a real burden but a pleasant one. When one becomes infected with the research virus, all tasks which do not permit an investigative angle can become irksome. There comes a time in life when robust health cannot be depended on. The editor's chosen bit of research is such that this is absolutely necessary. Hiking over mountain trails, back-packing, managing boats and apparatus on windy, snow-fed lakes, and living in camp near the snow-line, in sun, rain and snow makes delightful summer work, but it also makes demands. At least three more such summers are needed to complete the field work connected with the self-imposed task of a hydrobiological survey of the high lakes in northeastern Oregon which was stimulated and made obligatory by a grant from the National Society of the Sigma Xi a few years ago. To this pleasurable work, then, must the leisure time of some years be dedicated. Other pursuits, however pleasant, must wait.

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The increasing recognition which is given to articles appearing in NORTHWEST SCIENCE makes it highly desirable that much attention be given to the quality of the material appearing, and, to the make-up of the magazine. Trained instead of amateur editorship is almost imperative. It is no reflection on the ability of a scientist that he is unfamiliar with the established conventions used in printing articles in another science than that in which he specializes. The quality of paper now in use does not take halftone cuts well. Nor is it a paper with lasting qualities. Changes will add costs.

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Increased membership is one solution. One hundred new names would add fifteen or twenty percent to income. It would add only five per cent to publication costs. Increased advertising, if permanent, adds

but little to cost. If sought before the annual budgets of advertising firms are made up in the autumn, the number of our advertisers should be materially increased. The amateur solicitor can and does something to help this publication, but again this is a task for a professional.

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Marginal cuts used in the issue of November, 1936, have had some comments from our readers. They economize space for reading matter. They may also be some economy in cost. Column width for this magazine is two and one fourth inches, full page width is four and three-fourths inches. This could be where desirable five and three fourths inches. Halftones can of course be enlarged or reduced to fit space. Those marginal cuts were seven eighths of an inch wide.

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Photographic reduction in making line cuts is usually desirable. Inexperienced authors when they make drawings for illustrations frequently print letters too small to be read. To make sure yours can be read place your line drawing upside down before a camera with a ground glass focusing plate and focus to sharp image of required dimensions. Examine the image to see if the print is easily read. This little effort on part of the author will save much dissatisfaction. Cuts in this issue were reduced from drawings $6\frac{3}{8}$ and $3\frac{1}{4}$ inches diameter. Cuts are paid for by the square inch.

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Authors can readily calculate the number of pages required for publication if they will set the typewriter to 41 or 82 letter spaces. Lines of these lengths fill one or two columns widths. Allow fifty lines per column. Also make allowance for heading and for footnotes. Vigorous authoritative condensation usually adds to the quality of the article. Our membership can with little difficulty to themselves aid "our successor" by attention to such details as these.

T. LARGE.