shore birds. In this latter group there were a number of fresh-water forms characteristic of the ponds of the region, in addition to the ubiquitous unicellular green algae. Some of the more interesting algae found in a representative group of birds are listed in Table 1.

As a rule, more algae were present in birds shot during the spring than at any other time of the year. This probably reflected the greater diversity of algae normally present in the playas at that season. A scarcity of seeds and insects also may have caused the birds to ingest increased amounts of algae.

A microscopic examination of fecal material shortly after it was removed from the birds disclosed that *Gonium, Pandorina,* and at least four genera of desmids had passed through the alimentary canal as vegetative cells. Since many blue-green algae as well as a number of smaller unicellular green algae have no specialized resting stages they also must have passed through as vegetative cells. Viable filamentous green algae were never observed in any part of the digestive tract beyond the gizzard, although partially digested cells were often present. Filaments of *Spirogyra* and *Oedogonium,* present in the flasks after approximately one week, probably developed from zygotes.

Attempts to germinate intact *Chara* zygotes found throughout the guts of a coot and a blue-winged teal were unsuccessful. Neither motile yellow-green algae nor dinoflagellates were ever observed in cultures from any of the birds. The afore-mentioned three groups are known to have relatively complex cultural requirements, and the possibility cannot be ruled out that failure was due to unsuitable media or environmental conditions.

From a consideration of the rate of movement through the alimentary tract (*4*) and flying speed (*5*) of common migratory water birds, it seems reasonable to conclude that many fresh-water algae can be carried easily between lakes 100 to 150 miles apart. Cells or colonies in the caecum may be carried several times this distance.

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References and Notes

1. G. M. Smith, *Fresh-Water Algae of the United States* (McGraw-Hill, New York, 1950), p. 13.
2. F. E. Fritsch, *Structure and Reproduction of the Algae* (Cambridge Univ. Press, Cambridge, 1935), vol. 1, p. 360; J. H. Evans, *J. Ecol.* 46, 149 (1958).
3. E. Messikommer earlier reported finding identifiable algal cells in duck feces, but gave no indication that they were alive [*Hydrobiologia* 1, 22 (1948)].
4. P. D. Sturkie, *Avian Physiology* (Comstock, Ithaca, N.Y., 1954).
5. F. C. Lincoln, "Migration of birds," *U.S. Fish Wildlife Serv. Circ. No. 16* (1950).

17 April 1959

Effect of Light on Motility, Life-Span, and Respiration of Bovine Spermatozoa

Abstract. Exposure to light of bovine spermatozoa suspended in various media results in a progressive decline in metabolic activity followed by the premature death of the cells. The inhibitory and spermicidal effects of visible light resemble a photodynamic action; the photosensitizer is an intrinsic component of the cell.

The deleterious effects of short-wavelength radiation on spermatozoa have been examined in some detail by a number of workers (*1*). Wells and Giese (*2*), studying photoreactivation of ultraviolet-irradiated sperm of the purple sea urchin *Strongylocentrotus purpuratus,* observed that visible light was also very harmful to these cells, immotilizing them and rendering them incapable of fertilizing normal eggs. A later investigation (*3*) showed that glycine exerted a protective effect during exposure to visible light.

With the development of an artificial medium in which bovine spermatozoa can be maintained at room temperatures in a physiologically active state in vitro for several days (*4*), it has become possible to study experimentally the response of these cells to visible light.

The collection and processing of bovine semen was carried out essentially as described by Norman *et al.* (*4*). The sperm were suspended in a modified coconut-milk extender which contained, in final concentration, 15 percent coconut milk, 2.16 percent sodium citrate dihydrate, 0.068 percent dihydrostreptomycin sulfate, 0.031 percent sodium penicillin, 0.3 percent sulfanilamide, and 2.5 units of mycostatin per milliliter. The final concentration of the cells, determined with a hemocytometer, was between 10×10^6 and 15×10^6 cells per milliliter. Plastic vials containing the cell suspension were illuminated for varying periods on a glass plate 7 mm thick, placed 33 cm from a bank of two 40-watt white fluorescent bulbs. These lamps have a spectral range from 0.4 to 0.7 μ (*5*). The light intensity at the level of the sample vials was 300 ft-ca, as measured with a model 756 Weston illumination meter, and the ambient temperature was maintained at 26° ± 2°C during the experiment. The parameters used to determine the light sensitivity of the cells were the percentage of dead cells, as measured by the differential staining method of Campbell, Dott, and Glover (*6*); motility; and oxygen consumption, expressed as Z_{O_2}. Appropriate controls were kept in the dark.

Table 1 is a summary of the results of three replicate experiments on continuous illumination of the spermatozoa. These data indicate that exposure to light results in concomitant loss of mo-

Table 2. Effect of light on spermatozoa suspended in various media. Light intensity, 1400 ft-ca.

Exposure (hr)	Dead cells %		Motility	
	Light	Dark	Light	Dark
Krebs-Ringer phosphate				
10	56	30	2.0	3.5
60	63	39	0.5	2.5
*Krebs-Ringer phosphate plus antibiotics**				
10	74	38	0	3.5
60	100	52	0	3.0
0.85% NaCl				
10	89	32	0	4.0
60	100	68	0	2.5
0.85% NaCl plus antibiotics				
10	88	32	0	3.5
60	100	100	0	0
15% Coconut milk in Krebs-Ringer plus antibiotics				
10	51	22	0	4.5
60	100	34	0	3.0
Skim milk†				
24			0	3.0
60	95	36	0	2.5

* Same as in coconut-milk extender.
† Temperature, 5°C.

Table 1. Effect of light on spermatozoa suspended in various media. Light intensity, 300 ft-ca. Values are averages for three replicate experiments. Temperature during exposure, 26° ± 2°C.

Exposure time (hr)	Dead cells (%)		Motility*		Z_{O_2}	
	Light	Dark	Light	Dark	Light	Dark
Coconut-milk extender (10×10^6 to 15×10^6 cells/ml)						
24	50	32	0	3.0	0.4	5.34
72	100	36	0	3.0		
Coconut-milk extender and catalase (10 mg%) (10×10^6 to 15×10^6 cells/ml)						
24	36		2.0			
Coconut-milk extender (2×10^8 cells/ml)						
24	32	36	2.5	3.5		
72	73	31	1.0	3.0	0.0	3.5

* Motility ratings: 5.0, excellent; 4.0, good; 3.0, fair; 2.0, poor; 1.0, vibratory; 0.0, nonmotile.

tility and decrease in respiration of the sperm, followed by death of the cells. Apparently, with a more concentrated suspension of cells, sensitization is prolonged. The reason for this is not known at the present time. The protection afforded by the addition of catalase to the medium indicates that a photochemical oxidation producing H_2O_2 is occurring as a result of the radiation.

In order that this phenomenon may be defined as photodynamic action, it is necessary that there be some fluorescent substance present which can absorb radiant energy (7). The activated sensitizer transfers its energy to an acceptor, presumably a cellular protein, which then undergoes oxidation. To determine the source of the photosensitive agent, the effect of light on spermatozoa suspended in various media was tested. Since these cells will not survive at room temperature for a prolonged period of time, except in coconut-milk extender, it was necessary to modify slightly the experimental design and expose the samples to a higher light intensity (1400 ft-ca) for a shorter time. The results are shown in Table 2. The data clearly reveal that the photosensitive agent is not a constituent of any of the media tested or of the antibiotics which supplement them, although the latter may enhance the photochemical reaction. Comparison of Tables 1 and 2 indicates that the effect of radiation—presumably an oxidative process—is in accord with the Bunsen-Roscoe reciprocity law (7). Further, the effect is independent of temperature, as demonstrated by the results with the skim-milk diluent at 5°C. These are characteristics usually identified with a photosensitized oxidation (7).

On this basis, then, it can be concluded that there is a photosensitizer present within the sperm itself and that the radiation affects the cell directly. Calcutt (8), working with *Paramecium*, suggests that light has a direct action upon the test material, inducing a cellular change which facilities the photodynamic response. This phenomenon apparently involves cytoplasmic damage, as compared with the nuclear effects of ultraviolet radiation (9).

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References and Notes

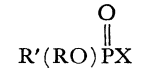
1. T. Mann, *The Biochemistry of Semen* (Wiley, New York, 1954), pp. 58–60.
2. P. H. Wells and A. C. Giese, *Biol. Bull.* 99, 163 (1950).
3. A. C. Giese and P. H. Wells, *Science* 115, 239 (1952).
4. C. Norman, C. E. Johnson, I. D. Porterfield, R. S. Dunbar, Jr., *J. Dairy Sci.* 41, 1803 (1958).
5. H. F. Blum and M. R. Matthews, *J. Cellular Comp. Physiol.* 39, 57 (1952).
6. R. C. Campbell, H. M. Dott, T. D. Glover, *J. Agr. Sci.* 48, 1 (1956).
7. M. T. Clare, *Radiation Biology* (McGraw-Hill, New York, 1956), vol. 3, chap. 15.
8. G. Calcutt, *J. Exptl. Biol.* 28, 537 (1951).
9. Preliminary attempts to delineate the light source into narrower spectral bands indicates that the photosensitive agent within the sperm has a maximal absorption in the blue. We thank I. D. Porterfield of the dairy husbandry department for furnishing us with bovine semen, the College of Agriculture, West Virginia University, for financial support, and C. E. Johnson for his technical assistance.

28 April 1959

Mechanism of Enzyme Inhibition by Phosphate Esters

Abstract. A theory for the rapid specific reaction of certain phosphorous-containing esters with many proteolytic enzymes based on the ability of phosphorous to form one additional bond relative to carbon is presented. A stable tetrahedral phosphate ester is compared with a labile tetrahedral orthocarbonyl ester and a relatively stable pentagonal enzyme-phosphate ester complex is compared with a pentagonal enzyme-carbonyl substrate complex. The latter complex is assumed to be the transition state in the enzyme-catalyzed reaction. If the theory is correct, it opens up the possibility of studying intermediates and transition states from the known structures of chemical inhibitors.

The specific reaction of a group of organic phosphate esters of the type



where R is an alkyl group and R′ an alkyl or alkoxy group, with a class of esterases and phosphoglucomutases has long been known. Some pertinent chemical facts concerning the inhibition reaction are: (i) The reaction products are the degraded fragment (X) and the enzyme, phosphorylated on the hydroxyl group of a serine residue (1). (ii) The reaction is stoichiometric with the number of enzymatic sites and not with the total number of serine residues. (iii) The reaction proceeds with a high velocity with all the enzymes for which the stoichiometric relationship holds. (iv) The phosphorylated enzyme is catalytically inactive.

The extreme velocity of the inhibition reaction contrasts with the rates of homogeneous solution reactions of the phosphate esters. Tetraethylpyrophosphate, for example, is remarkably stable in neutral aqueous solutions. Even di-isopropyl-phosphofluoridate is much more stable than acetic anhydride (2). Nonetheless, it reacts much more rapidly and specifically with suitable esterases.

In this report we wish to suggest that the action of the phosphate esters differs from that of most other enzyme inhibitors in that it is the unstable transition state of the enzyme-substrate complex which is imitated by the inhibitor in one of its stable combinations with the enzyme. More frequently, it is assumed that inhibitor action is effected by imitation of either the substrate itself or a *stable* combination of substrate with enzyme.

If our postulate is correct, one can understand why the side group specificity of the inhibitors is so different from

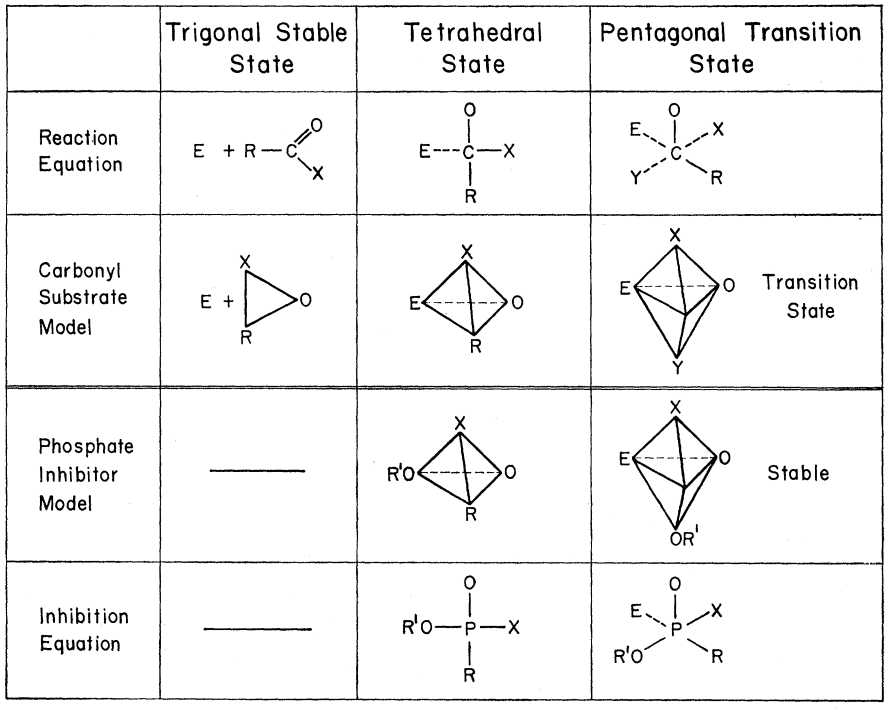


Fig. 1. Stereochemical representation of enzyme action.