and perhaps others) with the developing seeds of carrot and dill, and the results suggest that these insects are responsible for the natural occurrence of embryoless seeds in these species.

Lygus bugs are widely distributed and are known to feed on many of our native wild and cultivated plants. They reduce seed crop yields by causing bud blasting, as well as blossom and young fruit drop in alfalfa, beans, and cotton (*6, 8, 10*), seed spotting and pitting in the common and lima beans (*1, 4*), shriveled empty seeds in alfalfa (*2*), and reduced germination in beet, cotton, and guayule (*5, 7, 9*). As far as the writer is aware, a relationship between embryoless seeds in the Umbelliferae and Lygus bugs has not previously been observed.

In attempts to determine the cause of embryolessness, various possible factors, such as type of soil, locality, weather conditions, pollinating insects, and genetical influence, were studied but none of these seemed to have any bearing on the problem. Embryolessness was found to appear at random from year to year, with no correlation in regard to position on the plant or within the umbel (flower cluster). However, there was some indication that the seeds within a pair sometimes behaved similarly. Within a given sample embryolessness was present in seeds of all sizes. It was noted that embryoless seeds seemed to appear at a rather late stage of seed development, usually after the endosperm was more or less completely formed—that is, when it was white and firm. Very little embryolessness occurred early in the season at Yonkers, New York, but it was often quite prevalent in the midseason and early fall crops.

Various types of insects found visiting the flowers and developing fruits of various members of the Umbelliferae growing in Yonkers were caged with dill plants. Embryoless seeds almost invariably occurred, usually in very high percentages, on the plants or specific umbels which had been caged with Lygus bugs. Except for a few instances, no embryoless seeds were produced in either the control plants (caged insect-free) or in plants caged with other types of insects, such as ants, aphids, bees, Japanese beetles, lady beetles, and syrphid flies. The presence of a few embryoless seeds under these circumstances indicated either that the Lygus bugs, especially nymphs, were not effectively excluded or that other factors, perhaps other insects, may occasionally have an influence in producing embryolessness. In the open field where dill plants were exposed to all types of insects, the percentage of embryoless seeds ranged from 0 to 62%, while no embryolessness occurred in the seeds from umbels protected from insects by cages. In the case of plants caged with Lygus bugs throughout the period of flowering to the production of mature seed, the amount of embryoless seeds obtained ranged from 1 to 100%, with an average of 58%. There was some indication that contact with Lygus bugs at the time of flowering or shortly thereafter reduced seed yield, while contact for only 48 hr at later stages of seed development produced embryoless seeds.

These results establish that the feeding of Lygus bugs produces embryoless seeds in dill. Preliminary results with carrot were similar. Details of these experiments are appearing elsewhere.

References

1. Baker, K. F., Snyder, W. C., and Holland, A. H. *Phytopath.*, 1946, **36**, 493–503.
2. Carlson, J. W. *J. agric. Res.*, 1940, **61**, 791–815.
3. Flemion, F., and Uhlmann, G. *Contr. Boyce Thompson Inst.*, 1946, **14**, 283–293.
4. Hawley, I. M. *N. Y. (Cornell) agric. exp. Sta. Mem.*, **55**, 1922.
5. Hills, O. A. *J. agric. Res.*, 1943, **67**, 389–394.
6. McGregor, E. A. *U. S. D. A. tech. Bull.*, **4**, 1927.
7. Romney, V. E., York, G. T., and Cassidy, T. P. *J. econ. Ent.*, 1945, **38**, 45–50.
8. Shull, W. E. *Ida. agric. exp. Sta. res. Bull.*, **11**, 1933.
9. Smith, G. L. *Calif. agric. exp. Sta. Bull.*, **660**, 1942.
10. Sorenson, C. J. *Utah agric. exp. Sta. Bull.*, **284**, 1939.

A Metabolic Regulator in Mammalian Spermatozoa[1]

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The metabolic processes by which mammalian spermatozoa derive energy for motility have been a subject of investigation in this laboratory for some time. When the results of a series of studies (*8–15*) of the metabolism of bovine spermatozoa obtained from semen were compared with those of other investigators (*3, 4, 18, 19, 20*) who had studied epididymal spermatozoa, it became apparent that the metabolic pattern exhibited by bovine sperm cells from these two sources differed greatly. Since these workers (*3, 4, 18, 19, 20*) had used a wide variety of techniques and experimental conditions, a study was made (*7, 15*) of the metabolism of washed bovine epididymal spermatozoa in the calcium-free buffer-salts solution developed for ejaculated spermatozoa (*11*). Under these experimental conditions several metabolic differences between ejaculated and epididymal spermatozoa were observed. Those pertinent to the present discussion are:

(1) Most ejaculated spermatozoa have a vigorous endogenous respiration ($Q_{O_2} = 9$) (*10*). The addition of glucose results in a somewhat decreased rate of oxygen consumption (*8, 9, 12, 16*). In contrast, fresh epididymal spermatozoa have a comparatively low rate of endogenous respiration ($Q_{O_2} = 1–4$) while in the presence of glucose, respiration is appreciably greater ($Q_{O_2} = 2–6$) (*7*).

(2) Many specimens of ejaculated spermatozoa exhibit only a feeble Pasteur effect, *i.e.*, glycolysis in aerated media is almost as great as in the absence of oxygen (*8, 10, 14*). On the other hand, glycolysis by epididymal spermatozoa is 3–7 times faster under anaerobic conditions than it is in the presence of air (*7*). Certain specimens of ejaculated spermatozoa having a low endogenous respiration resemble epididymal sperm cells in that they exhibit a fairly strong Pasteur effect (*13, 14*)

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(3) Storage of excised epididymides alters their contained spermatozoa so that their metabolism more closely resembles that of ejaculated spermatozoa (*7*).

TABLE 1

AVERAGE RATES OF CATABOLIC REACTIONS IN BOVINE SPERMATOZOA*

	$-Z_{O_2}$		Z_L	
	Endogenous	+ Glucose	In air	In N_2
A. Epididymal	6	10	13	56
B. Ejaculated	20	16	20	25
C. Epididymal + regulator	20	22	20	35

* Z = mm³ gas exchange/100 million sperm cells/hr = 2.5 Q (*10*). Z_L = mm³ CO_2 equivalent to the lactic acid produced. The data represent averages of from 10 to more than 100 individual observations. Lactic acid was determined by the method of Barker and Summerson (*1*). Respiration measurements were made as described previously (*7, 11*).

Data summarizing these observations are included in Table 1, lines A and B, and in Table 2, line A. The data here are expressed in terms of Z values[2] instead of the more conventional Q values since cell counts are more conveniently made than dry weight determinations.

It is interesting to consider these metabolic data together with observations of motility. Epididymal spermatozoa with a Z_{O_2} of 6 move almost as vigorously as do ejaculated spermatozoa with a Z_{O_2} of 20. It appears, therefore, that the ejaculated spermatozoa are not as efficient in converting the energy of oxidative reactions to motion. The respiratory processes of the ejaculated spermatozoa are also less effective in depressing the rate of glycolysis.

A search for the cause of these differences in metabolic efficiency and for the phenomena described under (3) has led to the discovery of a metabolic regulator which is present in a bound (inactive ?) form in epididymal spermatozoa and which is apparently liberated in an active form soon after ejaculation. When small quantities of this regulator are added to epididymal spermatozoa an increased rate of respiration and aerobic glycolysis results immediately (Table 1, line C). The metabolism of epididymal spermatozoa in the presence of added regulator closely resembles that of ejaculated spermatozoa. Anaerobic glycolysis is usually depressed by the regulator, especially at higher concentrations. The response to a single level of the regulator is shown in Table 1, line C. Greater quantities depress respiration and glycolysis and abolish motility.

The biological activity of the regulator appears to result, at least in part, from its ability to uncouple exergonic oxidations from the phosphorylation reactions with which they are normally associated (*6*). The regulator resembles dinitrophenol in that it stimulates cellular respiration and aerobic glycolysis while preventing energy utilization (*2, 5, 14, 17*). We have devised an assay for the regulator based on its ability to stimulate the rate of aerobic fermentation by baker's yeast. Details will be presented elsewhere.

Using the yeast assay, it has been found that the regulator is present in a bound, inactive form in freshly ejaculated bull spermatozoa. During storage of semen at room temperature the substance is rapidly liberated from the spermatozoa into the seminal fluid in a water-soluble form which is still inactive for the yeast cell. The active substance is liberated from the water-soluble conjugate slowly during storage or more rapidly by mild alkaline hydrolysis. The substance liberated by hydrolysis is soluble in chloroform, ether, acetone, and petroleum ether. Alkaline hydrolysis is more effective in liberating the active form from the water-soluble conjugate than from the original bound form.

Fresh epididymal spermatozoa contain almost none of the free active regulator, but an appreciable quantity can be liberated by alkaline hydrolysis. When excised epididymides are stored in the refrigerator, the free form of the regulator is progressively released (Table 2). These results point to the likelihood that the increased rate of respiration of epididymal sperm following storage is brought about by the liberated regulator.

Furthermore, it seems probable that the free regulator liberated into semen after ejaculation is responsible for the higher rate of respiration of ejaculated spermatozoa as compared with epididymal spermatozoa.

We feel certain that the agent described plays an important role in the senescence of spermatozoa. If methods can be developed to prevent the liberation of the regulator or to counteract its effect once it is liberated, viable spermatozoa might be preserved for far

TABLE 2

EFFECT OF STORING EPIDIDYMIDES ON THE RESPIRATION OF THEIR CONTAINED SPERMATOZA, THE LIBERATION OF THE REGULATOR, AND THE RESPONSE TO ADDED REGULATOR*

Treatment	Time epididymides were stored at 5° C		
	3 hr	27 hr	51 hr
	$-ZO_2$		
A. Control	9	14.4	18.3
B. Plus regulator; 0.65 units/ml	20.3	20.3	22.4
	Units of regulator/10^8 sperm cells		
C. Boiled spermatozoa .	.04	...	0.7
D. Alkali-hydrolyzed spermatozoa	1.1	...	3.2

* One unit of regulator is the quantity required to give a half-maximum response in the standard yeast assay. Epididymal spermatozoa were washed with buffer-salts solution (*11*) before experimentation.

Respiration was measured in the presence of 0.01 M glucose.

Alkaline hydrolysis was carried out by suspending cells in 0.17 N NaOH and heating 10 min on boiling water bath.

[2] From the German "Zelle." The symbol was first used by Redenz (*18*).

longer periods of time than are now possible. The role of this regulator in sperm fertility is being studied.

References

1. Barker, S. B., and Summerson, Wm. H. *J. biol. Chem.*, 1941, **138**, 535.
2. Cross, R., Covo, G., Taggart, J., and Green, D. E. *J. biol. Chem.*, in press.
3. Henle, G., and Zittle, C. A. *Proc. Soc. exp. Biol. Med.*, 1941, **47**, 193.
4. Henle, G., and Zittle, C. A. *Amer. J. Physiol.*, 1942, **136**, 70.
5. Hotchkiss, R. D. *Adv. Enzymol.*, 1943, **4**, 153.
6. Lardy, H. A., and Elvehjem, C. A. *Ann. Rev. Biochem.*, 1945, **14**, 1.
7. Lardy, H. A., Hansen, R. G., and Phillips, P. H. *Arch. Biochem.*, 1945, **6**, 41.
8. Lardy, H. A., and Phillips, P. H. *J. biol. Chem.*, 1941, **138**, 195.
9. *Ibid., Amer. J. Physiol.*, 1941, **133**, 602.
10. *Ibid.*, 1941, **134**, 542.
11. *Ibid.*, 1943, **138**, 741.
12. Lardy, H. A., and Phillips, P. H. *J. biol. Chem.*, 1943, **148**, 333.
13. *Ibid.*, 1943, **148**, 343.
14. *Ibid.*, 1943, **149**, 177.
15. Lardy, H. A., and Phillips, P. H. *Nature, Lond.*, 1944, **153**, 168.
16. Lardy, H. A., and Phillips, P. H. *Arch. Biochem.*, 1945, **6**, 53.
17. Loomis, W. F., and Lipman, F. *J. biol. Chem.*, 1948, **173**, 807.
18. Redenz, E. *Biochem. Z.*, 1933, **257**, 234.
19. Windstösser, K. *Klin. Wchschr.*, 1935, **14**, 193.
20. Wolf, C. G. *J. Physiol.*, 1921–2, **55**, 246.

Lack of Depolymerase Effect on Desoxyribonucleic Acid in Living Cells

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Desoxyribonucleic acid depolymerase has been reported to remove ultraviolet light absorbing constituents and Feulgen stainable material, from nuclei in sections of tissue, and from nuclei of dead cells (*1, 2, 4*). There has been no reference in the literature, so far as the authors know, to the use of this enzyme on living cells.

In some experiments in this laboratory, it was found that the enzyme removed desoxyribonucleic acid from nuclei of chicken erythrocytes in smear preparations. After treatment with the enzyme, the nuclei failed to absorb ultraviolet light and did not stain with Feulgen's reagent. Addition of the enzyme to freshly obtained chicken blood, however, failed to affect the nuclei. This result suggested that perhaps living cells were unaffected by this enzyme when it was added to the extracellular fluid; results of further investigation showed that this is so with chicken erythrocytes and cells of Walker Carcinoma No. 256 of the rat.

Smears of chicken erythrocytes were fixed by immersion in 95% ethyl alcohol; they were then placed into fresh chicken blood to which had been added an equal volume of a solution of desoxyribonucleic acid depolymerase. The preparations were incubated at 37° C for periods of time up to 3.5 hr. In other experiments, suspensions of cells of Walker Carcinoma No. 256 of the rat in Ringer's solution were used in a similar manner. After incubation portions of the tumor cell-enzyme mixtures were transplanted into rats; if tumor growth followed it was assurance that the cells were living during the experiment.

The ultraviolet light (2654 A) absorbing material and the Feulgen stainable material in nuclei of the smears of both chicken erythrocytes and tumor cells were removed gradually and completely; however, there was no apparent effect on the nuclei of supposedly living chicken erythrocytes in the enzyme-blood mixture, or on the suspension of tumor cells. The tumor cells produced tumors after subcutaneous inoculation into rats; assuredly, they were living during exposure to the enzyme.

The enzyme attacked cells killed by heat, formaldehyde, alcohol, Carnoy's fixative, and ultraviolet light. Apparently the method of killing the cell makes little difference; it appears to be only necessary that the cell be dead for the enzyme to act.

Inability of the enzyme to act on the desoxyribonucleic acid of living cells might be explained by: a) absorption of the enzyme by cell constituents other than nucleic acid; b) antienzyme action; c) impermeability of cell membranes; d) inability of the enzyme to attack nucleic acid in the state that it exists in the living cell.

The possibilities that absorption and antienzyme activity prevented action of the enzymes on living cells were excluded by the fact that dead cells were acted upon by the same enzyme solution which failed to act on living cells. The assumption that membranes of the living cell are impermeable to depolymerase offers a plausible explanation for the lack of effect on living cells; however, it cannot be proved indisputably.

Whether or not other enzymes added to the exterior environment of living cells would fail to act on the respective substrates in cells is not known. Northrop (*3*), in 1926, reported that trypsin and pepsin were not taken up by cells of living organisms (earthworms, Euglena, yeast, meal worms, gold fish, and Fundulus), whereas, when the organisms died the enzymes were taken up rapidly from solution.

Desoxyribonucleic acid depolymerase did not act on nuclei of living chicken erythrocytes or of living cells of Walker Carcinoma No. 256 of the rat; the enzyme acted on these cells after they were killed. Lack of effect of the enzyme on living cells apparently was not because of adsorption of the enzyme or antienzyme activity, but may have been because of cell membrane impermeability or inability of the enzyme to attack nucleic acid in the state that it exists in the living cell.

References

1. Brachet, J. *Symposia of the Society for Experimental Biology. I. Nucleic acid.* Cambridge: University Press, 1947.
2. Catcheside, D. G., and Holmes, B. *Ibid.*
3. Northrop, J. H. *J. gen. Physiol.*, 1926, **9**, 497.
4. Stowell, R. E. *Stain Technol.*, 1946, **21**, 137.