Preliminary studies have not shown any obvious staining or morphological differences in chromatographed granules as compared with smears or centrifuged preparations. There was, however, an apparent increase in uniformity of population. Fig. 4 shows an electron micrograph of Harding-Passey granules separated by flowing chromatography.[3]

The adaptation of chromatography to particulates ranging from virus to mitochondrial and bacterial size provides another method for separation and characterization of the particulate components of the cell. Since columnar chromatography presumably exploits the surface and molecular configurations of particles rather than their mass or specific gravity, the method may provide a new attack on the problem of separating morphologically similar units possessing different physical or chemical surfaces.

TABLE 2

RELATIVE DOPA OXIDASE ACTIVITIES PER UNIT OF NITROGEN IN S91 MELANOMA FRACTIONS OBTAINED BY PARTIAL CENTRIFUGAL PURIFICATION FOLLOWED BY CHROMATOGRAPHIC SEPARATION

Fraction	$QO_2(N)$ Value			
	Exp. 1	Exp. 2	Exp. 3	Mean
1. Starting extract*	23.5	13.1	14.2	16.9
2. Sedimented granules† ...	79.5	31.4	27.0	46.0
3. Column eluate‡	225.1	130.6	147.5	167.7
4. Sedimented granules from eluate§	372.4	185.4	78.1	212.0

* This extract prepared essentially as described in text footnote 2 except for being cleared twice at approximately 1,000 × gravity.

† This granule suspension consisted of all the material sedimented by subjecting the cleared starting extract to a force of approximately 4,000 × gravity for 10 min. The resulting pellet was resuspended to the original volume with 0.9% NaCl.

‡ The 10-fold increase in enzyme activity of this fraction compared with the starting extract has a $P < .001$ and is thus highly significant statistically. It is not known at present whether removal of an inhibitor is involved in this increase or not.

§ The granules in the column eluate were sedimented in the same way and simultaneously with those obtained in fraction 2 from the starting extract. This pellet was also resuspended to its starting volume with 0.9% NaCl.

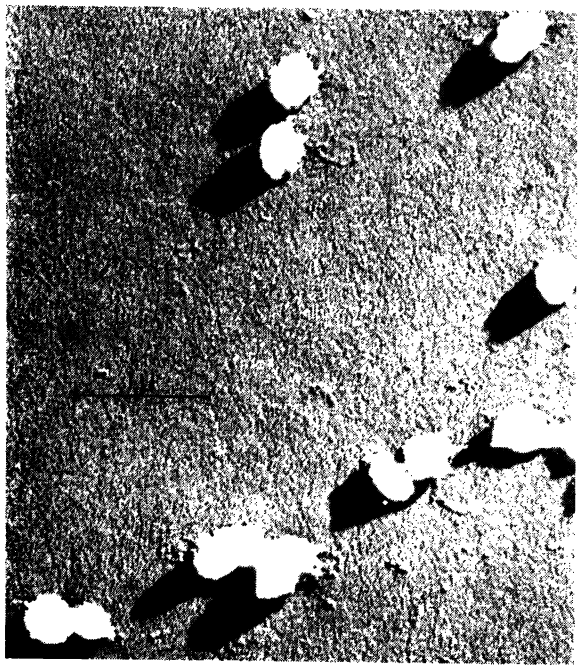

FIG. 4.—Electron micrograph of melanin granules from the Harding-Passey mouse melanoma obtained by chromatography. Gold shadowed at the approximate angle tangent 1/3. Magnification 8,000 ×. Enlarged to about 18,000 ×.

The adaptation of chromatography to particulates ranging from virus to mitochondrial and bacterial size provides another method for separation and characterization of the particulate components of the cell. Since columnar chromatography presumably exploits the surface and molecular configurations of particles rather than their mass or specific gravity, the method may provide a new attack on the problem of separating morphologically similar units possessing different physical or chemical surfaces.

References

1. DUBUY, H., WOODS, M., BURK, D., and LACKEY, MARY. *J. Nat. Cancer Inst.* Feb. 1949.
2. MASON, HOWARD S., KAHLER, HERBERT, MACCARDLE, ROSS C., DALTON, ALBERT J. *Proc. Soc. exp. Biol. and Med.*, 1947, **66**, 421–431.
3. RILEY, VERNON T. *Science*, 1948, **107**, 573–575.
4. STRAIN, HAROLD H. *Chromatographic adsorption analysis.* New York: Interscience, 1945.
5. WOODS, M., DUBUY, H., BURK, D., and HESSELBACH, MARIE. *J. Nat. Cancer Inst.* Feb. 1949.
6. ZECHMEISTER, L., and CHOLNOKY, L. *Principles and practice of chromatography.* New York: John Wiley, 1943.

Lygus Bugs in Relation to the Occurrence of Embryoless Seeds in the Umbelliferae

Florence Flemion

Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York

In fresh seeds of various members of the Umbelliferae family, quite often a considerable number—sometimes 50% or more—will not germinate, although they appear well filled and in good condition. Examination of many different lots of umbelliferous seeds (*3*) has shown that the fresh seeds which failed to germinate were without embryos while those containing mature embryos gave satisfactory germination. In these embryoless seeds the endosperm is present and from all appearances normal while the very small embryo which normally lies imbedded in the endosperm at one end of the seed is lacking. In no other family have such high percentages of embryoless seeds been reported; embryolessness, if present in other groups, is usually found in less than 1% of the seeds. The notoriously poor germination of umbelliferous seeds is evidenced by the rather low minimum germination standards set by the Federal government—55% for carrot and celery and 60% for parsley and parsnip. Evidence is presented in this paper that embryolessness occurs following the caging of Lygus bugs (*Lygus oblineatus* Say[1]

[1] Identified by Dr. Reece I. Sailer of U. S. National Museum.

[3] Electron microscopy by Dr. Herbert Kahler and Mr. Bolivar J. Lloyd, National Cancer Institute.

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]

Henry A. Lardy, D. Ghosh, and G. W. E. Plaut

Department of Biochemistry, University of Wisconsin, Madison

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*)

[1] Published with the approval of the director of the Wisconsin Agricultural Experiment Station. This work was supported in part by a grant from the Rockefeller Foundation to C. A. Elvehjem and by the Badger Breeders Cooperative.