needs the entire molecule of coenzyme I or coenzyme II and can not synthesize it from nicotinamide. The dysentery organism when grown on a synthetic medium with nicotinamide can synthesize a compound (or compounds) identical with or an adequate substitute for one or more of the coenzymes needed by H. influenzae.²

By means of the Thünberg technique, Lwoff and Lwoff have shown⁴ that H. parainfluenzae cells grown in a medium which is deficient in the "V" factor (presumably coenzyme I or coenzyme II) have their respiration markedly stimulated by the addition of coenzyme I or coenzyme II to the Thünberg tubes. A similar stimulation of oxygen uptake could be demonstrated by means of the Warburg technique. This stimulation disappears when the cells are grown in a medium containing larger amounts of "V" factor, *i.e.*, the addition of coenzymes to the Thünberg tubes does not increase the respiration.

We have performed similar experiments in which we used dysentery bacilli grown on a medium containing suboptimum amounts of nicotinamide. We found that the rate of reduction of methylene blue is greatly increased not only by the addition of coenzyme I, but also by the addition of nicotinamide or nicotinic acid to the Thünberg tube, as shown by the following protocol. This effect of nicotinamide and nicotinic acid has not previously been reported for any system. The cells (Strain D76) were grown 24 hours in synthetic medium containing 0.005γ nicotinamide. The cells from 100 cc of medium were collected in the centrifuge and suspended in M/20 buffer (pH 7.4). The cells were again centrifuged and suspended in 34 cc of buffer. Two cc of this suspension was added to each tube. Each tube also contained 0.30 cc of a 2 per cent. solution of glucose and 0.50 cc of a solution of methylene blue containing 100γ of methylene blue per cc. The nicotinamide and nicotinic acid solutions contained 100y per cc. The cozymase solution contained 300γ per cc and was 40 per cent. pure. Smaller quantities of a pure cozymase preparation from Euler's laboratory showed the same effect. Water was added to the controls to make the dilution the same in all cases. The tubes were equilibrated 10 minutes at 37° before tipping.

TABLE 1

Tube	Control	1	2	3
Nicotinamide	0	0.15 cc	0	0
Nicotinic acid	0	0	0.15 cc	Ŏ
Cozymase		0	0	0.15 c
Time (in sec.)	460	170	170	200

If we consider the ratio of the time of reduction control/nicotinamide, we find that the ratio decreases as the amount of nicotinamide in the *medium* is increased until with cells grown in a medium containing

4 Ibid., 360-73.

 0.020γ per cc it is about one. Thus at 0.003γ , the ratio is 3.8; at 0.005γ , 2.6; at 0.007γ , 1.8; at 0.010γ , 1.5; and at 0.020γ , 1.1. In controls containing nicotinamide, etc., but not glucose, reduction was much slower, 600 seconds or more. In controls which contained cells alone the methylene blue was reduced even more slowly or not at all. Nicotinamide, nicotinic acid or cozymase and glucose gave no reduction of methylene blue in the absence of cells.

The same stimulation of respiration by nicotinamide, etc., was found when oxygen uptake was measured by the direct Warburg method. It should be emphasized that various strains of the dysentery organism differ in their nicotinamide requirements. Further work in an attempt to elucidate the mechanism of this codehydrogenase action of nicotinamide is in progress in this laboratory.

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SOMATOPLASTIC STERILITY IN MEDICAĜO SATIVA¹

THE collapse of ovules during the early stages of post-fertilization development frequently occurs in alfalfa, particularly after self-pollination.² Histological study shows that the collapse follows abnormal growth of the somatic tissue adjacent to the embryo sac. Since local hyperplasia of the maternal structures appears to be the essential feature of the developmental course leading to collapse, the term somatoplastic sterility is here proposed for a type of seed failure believed to occur in many plants.

The reproductive process in angiosperms may fail at any stage between fertilization and maturity of the seed. The early stages are of critical importance for survival of the ovule in alfalfa. Although somatoplastic sterility is not necessarily limited to this period, it is in young ovules that one would expect it to be manifested in simplest form. The case is of interest, therefore, as affording a point of departure in defining the significant histological features and in discovering the cause of a rather obscure, although probably widespread, form of sterility in plants.

The embryo sac in the mature ovule of alfalfa is surrounded by two integuments. The inner integument, which is composed of two layers of cells, lies in direct contact with the embryo sac except at the chalazal end where a few disintegrating cells, remnants of

¹ Papers from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin No. 248. The authors desire to express their appreciation for financial aid from the University Research Committee and for assistance from WPA project in the Natural Sciences No. 8649.

² Brink and Cooper, Proc. Nat. Acad. Sci., 24: 497-499, 1938.

the nucellus, are found. Shortly after fertilization active cell division is initiated in the integuments as well as in the endosperm mother cell and the zygote. The cells of the inner integument divide longitudinally so that this tissue normally continues to have two layers of cells at the later stages of development. The cells adjacent to the endosperm are tapetal-like in appearance.

In material collected 48 to 72 hours after pollination in which the collapse of fertile ovules is frequent the cytoplasm of the cells immediately adjacent to the endosperm becomes finely vacuolate and densely staining, and the resemblance to tapetal cells is gradually lost. This condition, which makes its first appearance on the funicular side of the ovule in the region of the vascular bundle, is often seen before any impairment of the embryo sac becomes evident. Breakdown of the endosperm follows, beginning in the chalazal region and progressing toward the embryo. At this stage there is extensive meristematic activity in the chalazal portion of the inner integument. Through transverse division of the cells this tissue may come to be three to five layers in thickness. The ovule may continue to enlarge for several hours, even after the endosperm has broken down, but its growth soon ceases.

The frequency of collapse of fertile ovules in alfalfa is much higher following self-pollination than after outcrossing to unrelated plants. In a representative experiment involving seven individuals on which the two types of matings were made under strictly comparable conditions the average values were 34.4 per cent. and 7.1 per cent., respectively, in the interval up to 144 hours after pollination.² The relatively high survival of ovules containing hybrid, as compared with inbred, embryos and endosperms affords a clue to the cause of somatoplastic sterility.

Fertilization initiates development of the embryo and the endosperm and stimulates mitosis in the surrounding somatic tissue. The ovule, quiescent prior to gametic union, suddenly springs into active growth. Further development of these structures is largely dependent upon food moved in from other parts of the plant, the visible reserves immediately at hand in alfalfa being very limited in amount and quickly disappearing after fertilization. The critical factor for survival at this stage seems to be the manner in which the translocated food is shared between the endosperm, on the one hand, and the inner integument, on the other. The partition of nutrients appears to depend upon the rate of growth inside and outside the embryo sac. It may be assumed that the synthetic processes are essentially alike in the different tissues concerned, and hence, that the same raw materials are in concur-Under these conditions of parallel rent demand. growth the available foods will be shared between the inner integument and the structures within the embryo

sac in proportion to the velocity with which growth is occurring in the respective tissues. Continued development of the ovule following fertilization demands a balance in rate of growth between the endosperm, which is the dominant tissue within the embryo sac, and the adjacent maternal tissue which will insure the nourishment of both. If, during early development, the balance is upset by failure of the endosperm to keep pace with growth in the extensive surrounding tissue the endosperm starves and, eventually, the ovule collapses.

Following hybridization in alfalfa, the rate of growth of the endosperm, as measured by the number of nuclei, is found to be significantly higher than after selfing. The two classes of embryos, on the other hand, grow at only slightly different, and much lower, rates. As mentioned above, only about one fifth as many fertile ovules collapse after crossbreeding as after selfing. The initial conditions in the ovule outside the embryo sac being alike in the two cases, it seems clear that the higher survival following crossing is the result of the more active growth of the hybrid Conversely, following self-fertilization, endosperm. the rate of growth of the endosperm is frequently so low that the balance soon shifts in favor of the integument. The hyperplasia we have described then arises, causing collapse of the endosperm and, eventually, terminating development of the ovule.

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