lated from cardiac blood at this time. Serum from Chinese hamsters infected 20 days previously was capable of forming, *in vitro*, circumoral precipitates on adult female worms isolated from the intestine of the golden hamster.

In a parallel study (*6*), it was found that animals which had been infected for 14 to 26 days exhibited severe generalized myositis. Although the latter condition doubtless constitutes a stress situation for the host, it does not appear to be reflected in changes in adrenal lipid within the time limit of this study. Hematoxylin- and eosin-stained sections of the adrenals exhibited no evidence of pathological changes during the course of the experiment and beyond (to 112 days of infection). Adrenal weights (*6*) bore no obvious relationship to the status of the infection. It is possible that stress brought about by the migratory phase of the infection would be mediated or reflected in other endocrine changes or that measures of adrenal response other than sudanophilic substance would resolve this apparent duality of physiological response. Data on the Chinese hamster response should constitute a useful adjunct for comparative study in connection with the studies of the singular adrenal responses of the golden hamster in stress situations.

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Ultracentrifugal Determination of Molecular Weight of Myosin by the Archibald Procedure

Attempts to determine the molecular weight of myosin from ultracentrifugal sedimentation and diffusion data have not, so far, been successful. This failure is due not only to the fact that there has been no agreement about the values for the sedimentation and diffusion constants but is aggravated by the finding of Parrish and Mommaerts (*1*) that the sedimentation constant, after the usual corrections, still depends not only on the protein concentration but also on the temperature and rotor speed. These kinetic anomalies, which we have confirmed in the present work, are entirely unexplained and seem to invalidate any efforts to determine the molecular weight in the traditional fashion.

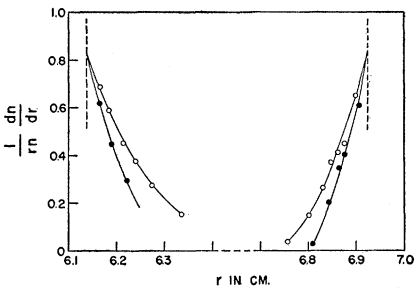

Fig. 1. Archibald plots for the ultracentrifugal sedimentation of myosin. Protein concentration, 0.5 percent; temperature, 5°C; rotor speed, 4196 rev/min; duration, 24 and 42 hours. The parameter $\frac{1}{rn} \times \frac{dn}{dr}$ is plotted against the distance in the cell, r; the curves, extrapolated toward the meniscus and the bottom of the cell (vertical dotted lines), indicate the values for δ.

It seemed advisable, therefore, to attempt a determination with methods which have an equilibrium rather than a kinetic basis. Since equilibrium centrifugation would be impracticable because of the long duration, we have followed the direction indicated by Archibald (*2*), in which the approach toward sedimentation-diffusion equilibrium is investigated. We have made the required measurements in a Spinco ultracentrifuge equipped with an optical system for the observation of Rayleigh interference fringes. These measurements, as explained by Archibald, are intended to estimate a parameter

$$\frac{1}{rn} \times \frac{dn}{dr} = \delta$$

in which n is the protein concentration and r is the distance from the center of rotation, from which the molecular weight is obtained by the relation

$$\delta = M(1 - V\rho)\omega^2/RT$$

In 13 separate experiments (for example, Fig. 1) on four different crystallized myosin preparations with moderate variations of concentration, rotor speed, duration, and temperature, the molecular weight was consistently found to be 382,000 with individual variations within ± 20,000. In all instances, the same molecular weight was obtained from extrapolation toward the top and toward the bottom of the cell. In one more elaborate experiment, in which, at 4196 rev/min and at 5°C, exposures were taken every 12 hours for 3 days, the molecular weight was found to be 385,000 ± 4000 for the top of the cell and 375,000 ± 4000 for the bottom.

These values are in definite disagreement with all results published so far, but they seem to rest on a much better experimental and theoretical foundation (*3*). It may be pointed out that a figure of 380,000 to 400,000 corresponds to the presumable weight of the myosin moiety of the 504,000 g of actomysin which, according to Nanninga and Mommaerts (*4*), reacts stoichiometrically with 1 mole of adenosine triphosphate. Hence, the myosin molecule has one active center for this interaction (*5*).

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3 September 1957

Effect of Kinetin and Gibberellic Acid on Excised Anthers of Allium cepa

The process of meiosis and the subsequent formation of haploid male gametes is one of the most important events in the life history of an angiosperm. Recently some attempts have been made to cultivate anthers on nutrient media and to follow the course of meiosis and pollen development in order to understand their physiology and biochemistry. Anther culture technique not only seeks to throw light on the mechanism of meiosis but can be of great help in solving problems of cytology and of growth and differentiation.

So far, the best development of pollen *in vitro* has been obtained with anthers of *Trillium erectum* with the help of 25 to 50 percent coconut milk (*1, 2*). Linskens (*2*) believes that the beneficial effect of coconut milk is due to its nucleic acid content. Anthers excised earlier than pachytene or even diplotene-diakinesis generally failed to undergo meiosis in culture media. It is believed that certain substances are transported to the anthers from the flower or from some other region of the plant at this stage and that these substances are responsible for the development of the anthers.

Recent studies have shown that two new growth-promoting substances, kinetin (*3*) and gibberellic acid (*4, 5*), have a very marked effect on the growth of

plants and plant tissues. In this report the results of some experiments on the effect of kinetin and gibberellic acid (*6*) on the growth and development of excised anthers of *Allium cepa* are presented (*7*). To the best of my knowledge, no other report is known where excised anthers have been grown from leptotene to one-celled microspores with such a high degree of survival as is presented here.

White's modified basic medium (1 percent sucrose and 2 mg of indoleacetic acid per liter) was used throughout. Kinetin (aqueous solution; dissolved with the help of 1*N* hydrochloric acid; concentrations used were 0.01, 0.05, 0.1 and 0.15 mg/lit) and gibberellic acid (dissolved in absolute ethyl alcohol; concentrations used were 1, 5, 10 and 25 mg/lit) were incorporated in the basic medium.

The plants were raised under normal field conditions during March and April. Prior to inoculation, one out of the six anthers (all the six anthers were at approximately the same stage of development) was removed and an iron-aceto-carmine squash was prepared to check the stage of development. The other five anthers were then sterilized in an 8 percent calcium hypochlorite solution for 3 minutes, washed with sterile, double-distilled water, dissected, and inoculated under aseptic conditions. In all, 1050 anthers (210 cultures of five anthers each) were cultured. All the cultures were exposed to diffuse laboratory light and stored at 20° to 25°C.

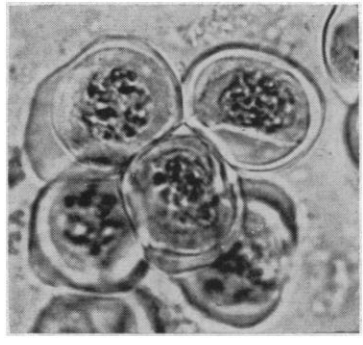

Fig. 1. Anther cells excised at leptotene-zygotene (×540).

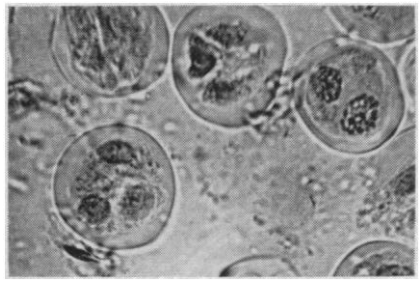

Fig. 2. Anther cells excised at leptotene-zygotene and cultured for 2 days in White's medium with 0.05 mg of kinetin per liter. Dyads and tetrads (about ×500).

In White's medium, anthers excised at zygotene, leptotene-zygotene (Fig. 1), and metaphase I degenerated within 2 days. Their cell walls thickened, and their contents were gradually used up. In double-distilled water and in 0.8 percent agar, anthers excised at leptotene-zygotene degenerated and their contents were used up within 6 days of culture.

In White's medium supplemented with kinetin, the optimum concentration of kinetin (Fig. 2) proved to be 0.05 mg/lit, and in this medium anthers excised at leptotene-zygotene showed, after 9 days, 10 percent mother cells in metaphase I, 40 percent in metaphase II, and 45 percent tetrads, while 5 percent remained undeveloped.

In White's medium with gibberellic acid, optimum development was seen at 5 mg/lit (Fig. 3), and after 2 days there were 12 percent undeveloped mother cells, 32 percent dyads, 14 percent cells in metaphase II, and 42 percent in telophase II; after 6 days 87 percent of these had formed tetrads, while 13 percent had degenerated.

In anthers excised at leptotene-zygotene, and cultured in White's medium supplemented with kinetin (0.05 mg/lit) and gibberellic acid (5 mg/lit), only 5 percent of the microspore mother cells remained undivided, 20 percent formed dyads, 17 percent showed anaphase II, and 58 percent formed tetrads within 2 days (Fig. 4). This was as good as, or slightly better than, the development observed *in situ*. Within 4 days all the cells had formed tetrads. The anther filament was removed during inoculation, but the short portion attached to the base of the anther grew to about 1 mm in several cases and showed two to three small fingerlike processes at the lower end.

Thus *Allium cepa* anthers excised at leptotene-zygotene or even at leptotene grew satisfactorily in media containing kinetin and gibberellic acid, or both, and produced tetrads and one-celled microspores. In all other plants investigated so far (*1*), anthers excised at this stage failed to develop. It is known that kinetin induces cell division, but there is no report that gibberellic acid induces cell division except a recent short report by Sachs and Lang (*5*), who report that "gibberellin causes a great increase in the number of cell divisions in the subapical region of nonvernalized biennial *Hyoscyamus niger* rosettes, thus proving that gibberellin may function as a regulator not only of cell elongation, but also of cell division." The fact that gibberellic acid also causes cell division (as shown here) in addition to cell elongation, is a further evidence in favor of its being an auxin. The occurrence of gibberellinlike substances has been recently demonstrated in *Echinocystis* seeds (*8*).

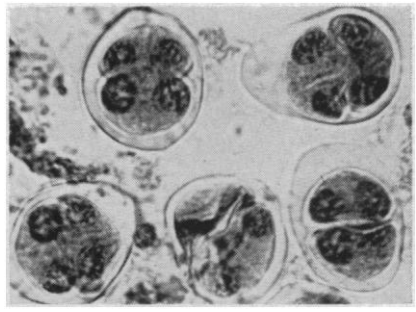

Fig. 3. Anther cells excised at leptotene-zygotene and cultured for 2 days in White's medium with 5 mg of gibberellic acid per liter. Late telophase II (about ×500).

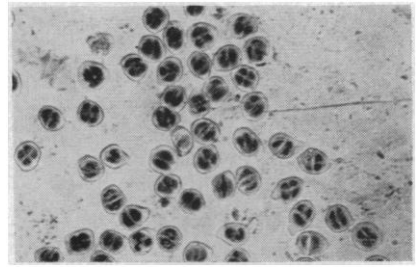

Fig. 4. Anther cells excised at leptotene-zygotene and cultured for 2 days in White's medium with 0.05 mg of kinetin and 5 mg of gibberellic acid per liter. Mostly tetrads, a few dyads (about ×130).

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