Islam, M. S. and J. Weijer. Sex hormones in Neurospora crassa.

Sex hormones (i.e., sex- and fertility-inducing substances) hove been isolated from cultures of crosses between Em A and Em a as well as from the homokaryons of Em A ond Em a of N. crassa. For the extraction of sex- and fertility-inducing substances from on established cross between wild type strains with on Emerson genetic background, 15 ml of sterilized liquid crossing medium were poured into a single Petri dish (size 100 x 15 mm.) in which 5 sheets of sterilized filter paper (#1, 9 cm.) were placed for the support of mycelial growth. Inocula of both Em A and Em a were mode simultaneously on the filter paper close to one another and the culture was incubated at a temperature of 23-26°C for three weeks, by which time free ascospores could be detected. A similar procedure was used for single strain cultures (either Em A or Em o), except for the fact that minimal medium was used.

Extraction was mode after three weeks of incubation of the cultures. The filter papers (50 X 5 of them) with the adhering mycelia were smked in 3000 ml. of sterilized distilled water for about two hours under occasional stirring with a gloss rod without disturbing the integrity of the filter paper and the adhering mycelia. Subsequently, the water was decanted ond the filter papers with the adhering mycelia were soaked again in on additional 3000 ml. of sterile distilled water. In total, 6000 ml of water-extract were obtained in this manner. The water-extract was then filtered through a filtering apparatus composed of 8 layers of cheese cloth together with a thick gloss wool pad ond, finally, 3 layers of filter paper #1.

Five grams of activated charcoal (Norit-A) were added to 500 ml. of the water extract. The mixture was agitated for a few minutes after which it was kept at room temperature for about 3 hours. Subsequently, as much water as possible was decanted without disturbing the charcoal sediment. The remaining water together with the charcoal was then filtered through filter paper #1 and the charcoal so collected was dried at room temperature. 300 ml. of chloroform (reagent grade) were added to the dried charcoal ond agitated vigorously for a few minutes, after which the mixture was kept at room temperature for about 3 hours with occasional and mild agitation, after which the chloroform mixture was filtered twice through 5 layers of filter paper #1. This extraction procedure was repeated once more and, finally, about 500 ml. of chloroform extract was obtained in this manner. The extract was then evaporated to dryness under vacuum suction. The residue, consisting of a

thin film of an oily substance was redissolved in 15 ml. of chloroform, collected in a specimen vial and kept under refrigeration.

The extracts were tested for their biological activity by dispensing 0. 1 ml. (2 µgm. dry weight) of extract on a small triangular piece of filter paper (#1) placed in the center of a test plate. The test plates contained 20 ml. of Westergaard's crossing medium and were simultaneously inoculated with Emerson A and Emerson a. Each experiment was repeated at least 5 times. The crosses were allowed to grow at room temperature (23-26°C) for three weeks. Observations were made at 7-day intervals. In addition, the extracts were tested on single strain cultures of either Em A or Em a grown on solidified minimal medium. The control consisted of the addition of a small triangle of filter paper onto which 0. 1 ml of chloroform was pipetted.

For biochemical tests, the extracts were purified. The method followed was basically that of Folch et al. (1957) for the purification of lipids. Re-distilled water (0.2 ml. by volume) was added to the final extract with thorough mixing and the mixture was allowed to separate into two layers for 15-30 minutes, after which the upper layer (water) was siphoned off care fully and discarded. The process was repeated once more, after which the solvent layer was stored under refrigeration.

The extracts were found to possess the following biological properties: (1) The extracts, when applied to cultures of single strains of Em A or Em a, induced a ten-fold increase in the number of protoperithecia and protoperithecia-like bodies developed by strain Em A; no increase was noted when Em a war used as the tester strain. (2) The extracts, when applied to crosses between sterile and wild type strains, improved the fertility (as measured by the number of perithecia with spore content) of some sterile strains of mating type A but not of a sterile strain of mating type a, when compared to untreated controls. (3) The extracts, when applied locally to a fertile cross (Em A x Em a), gave a five- to ten-fold increase in the number of mature perithecia developed by the cross. In addition, the extract caused a chemotactic response in terms of localized development of perithecia on the treated filter paper. (4) In an isolated case, the extract from a cross Em A x Em a was found to induce 'selfing' of an Em A strain, thereby giving rise to A, a, bisexual (self-sterile) and sterile progeny. No perithecia were observed in control cultures t reated with chloroform only. In all cases the plating test for bacterial and fungal contaminants of the extract remained negative.

Biochemical investigations using thin-layer chromatography (3:1 chloroform-benzene) indicated that two substances in the cross extract (Em A x Em a) moved with the solvent front. Chromatography of each of the single strain extracts (Em A or Em a) yielded only one biologically active spot moving with the solvent front. The chromatography spots yielded a lipid-positive color test with spray reagents. Ultraviolet spectrometry, infrared spectrometry, nuclear magnetic resonance, mass spectra determinations and microanalysis of the extracts of single strains (Em A and Em a) characterized the sex-inducing substances to be open-chained, unsaturated, and possibly branched hydrocarbons with a molecular weight of 354-372 (mating typ a) and 344-357 (mating type A). All tests confirmed a highly satisfactory degree of purification of these substances by the methods employed.

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