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Effects of Carcinogenic Agents on *Paramecium caudatum*.

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Recently Mottram¹ has shown that when cultures of *Paramecia* were submitted to long exposures of 3:4 benzpyrene, organisms appeared which were abnormal in size, shape and structure, etc. Such organisms, when isolated and placed in normal culture media, divided and gave rise to populations of abnormal *Paramecia*. The following account deals with experiments undertaken to learn whether other known carcinogenic agents would produce effects similar to 3:4 benzpyrene on *Paramecium caudatum*, and particularly whether these hydrocarbons would exhibit any differences in the rapidity and potency of their actions, such as Shear² and others observed in mice.

The carcinogens employed in these investigations were methylcholanthrene* (0.1% to 1%), Scharlach red (0.1% to 1%) and 3:4 benzpyrene (0.1% to 1%, and more dilute concentrations varying from 1 part carcinogen in 1000 parts of medium to 1 part in 100,000 parts of medium). These were finely suspended in rich hay infusion cultures of *Paramecium caudatum* in Syracuse dishes. Subcultures of both experimental and control animals were made at regular and identical intervals to prevent any contamination on the part of the medium. Similarly, organisms were frequently fixed on coverslips, stained and mounted for cytological study.

Results. In all of the experiments similar abnormalities appeared amongst the treated cultures. These abnormalities took the form of swellings, vacuolizations of the cytoplasm, and particularly blisterings of the pellicle. Monster formations, such as Mottram described, were not seen. Organisms exhibiting these abnormalities, even when isolated in normal media, became sluggish and misshapen, and soon died as noted below.

Differences in the potency and rapidity of action of the carcinogenic agents were indicated by the time of appearance of abnormal-

¹ Mottram, J. C., *Nature*, 1940, **145**, 184.

² Shear, M. J., *Am. J. Cancer*, 1936, **26**, 322.

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ities, as well as by the relative number of abnormal organisms present. Thus, organisms growing in methylcholanthrene exhibited abnormalities within 30 to 32 days regardless of the different concentrations used. Here only a few organisms were affected. Survival following this treatment averaged 5 to 8 days. Scharlach red yielded almost identical results. However, 3:4 benzpyrene proved to be more effective. *Paramecia* growing in this hydrocarbon exhibited abnormalities within 12 to 14 days in all of the concentrations employed. These abnormal organisms rarely survived for more than 5 days. Furthermore, the number of organisms affected was considerably greater than either of the other agents employed. Cytological studies of abnormal organisms that were fixed and stained, gave no indication that either the macronucleus or micronucleus were affected or involved.

Conclusion. These results indicate that exposure of *Paramecium caudatum* to the carcinogens employed in these experiments, causes the production of certain abnormalities regardless of the different concentrations of the carcinogenic agents used. The order of potency for these carcinogens, as demonstrated by the time of appearance of abnormalities, is as follows: 3:4 benzpyrene; methylcholanthrene, and Scharlach red.

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Cell-Free Enzymes of *Azotobacter vinelandii*.*

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Bach and coworkers¹ have reported nitrogen fixation by a cell-free extract of *Azotobacter* cells. Roberg² was unable to confirm their results. The possession of a cell-free nitrogen-fixing enzyme system would greatly facilitate studies on the mechanism of nitrogen fix-

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¹ Bach, A., Jermoljeva, Z., and Stepanjan, M., *Compt. rend. acad. sci. (U.S.S.R.)*, 1934, **1**, 22.

² Roberg, M., *Jahrb. f. wiss. Bot.*, 1936, **83**, 567.