A T4-Bacteriophage Reversion Test for the Naturally Occurring Mutagen Present in Bracken Fern (*Pteridium aquilinum*)

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Previous work had shown that certain bracken extracts caused dominant lethal mutations in *Drosophila* and gave positive tests in a mammalian mutagen test (Evans, Barber, Jones & Leach, 1968, 1969). These fractions also contained the agent responsible for bracken poisoning of cattle and carcinogenic activity in small animals (Evans & Mason, 1965). To facilitate isolation of the active principle(s) a more rapid and sensitive test was desirable.

Mutant T4 bacteriophages produced by certain classes of mutagens can be induced to revert to the wild-type by the application of some other mutagenic agents (Freese, 1959). Thus, by using a T4 rII mutant bacteriophage, which will not lyse an *Escherichia coli* strain with a λ prophage, it might be possible to make it revert to the wild-type by using an unknown mutagen. The revertant r⁺ genotype could then be detected in the presence of the original rII mutant by using *E. coli* KB (λ) as indicator.

Nutrient agar plates were inoculated by spreading 3ml of sloppy nutrient agar (Oxoid; 1.3g/100ml) containing 0.1ml of a suspension of *E. coli* K (overnight growth, aerated in nutrient broth at 37°C), 0.1ml of a suspension of *E. coli* B (similarly grown, but diluted 1:10 with nutrient broth) and 0.1ml of stock T4 rII bacteriophage mutant (titre approx. 4×10^7 /ml); a control plate is inoculated by omitting the bacteriophage. The plate was divided into two halves and a hole (4mm diam.) gouged in each. An aqueous solution of mutagen or bracken extract (0.1ml) was placed in the holes; the plates were then left (for 2h at 4°C) to allow diffusion of the mutagen while the growth of bacteria and bacteriophage is inhibited. They were incubated overnight (37°C) and inspected. Results and interpretations can be divided into three categories.

(i) Substances that do not cause reversion of the rII mutant: bacterial growth extends evenly across the plate, and spontaneous revertants appear as a number of small plaques spread evenly across the surface.

(ii) Mutagens that cause reversion: a lysis zone is seen around the hole, the area being dependent on the nature of the mutagen and its concentration.

(iii) Mutagens that are also bactericidal or bacteriostatic: the clear area in the vicinity of the hole is caused by bacterial inhibition, as is confirmed by the control plate. Around this area there

sometimes occurs a ring of bacterial growth, possibly because the host cells have mutated to make them bacteriophage-resistant, or the bacteriophages themselves are inhibited in this region. Outside this circle occurs the lysis zone, where the purely mutagenic effect is strongest.

The test gives a positive response with about 1mg of the base-analogue-type mutagens, and with 0.1mg of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. The bracken mutagen, eluted from a single spot on a t.l.c. plate, was active at a concentration of 0.25mg. The method is useful, because of its speed and sensitivity, in following the chemical fractionation of this active principle.

Results obtained with this test and the possible mode of action of the mutagen will be discussed.

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Evans, I. A., Barber, G. D., Jones, R. S. & Leach, H. (1968). *Rep. Br. Emp. Cancer Campn.*, p. 416.

Evans, I. A., Barber, G. D., Jones, R. S. & Leach, H. (1969). *Rep. Br. Emp. Cancer Campn.*, p. 446.

Evans, I. A. & Mason, J. (1965). *Nature, Lond.*, **208**, 913.

Freese, E. (1959). *Proc. natn. Acad. Sci. U.S.A.* **45**, 622.

Isolation of an Active Principle from the Bracken Fern that is Mutagenic, Carcinogenic and Lethal to Mice on Intraperitoneal Injection

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The principle(s) in bracken that cause bracken poisoning of cattle was extracted from the dried plant into boiling ethanol (Evans, Evans, Thomas, Watkin & Chamberlain, 1958); thiaminase, which is also present in bracken (Evans, Jones & Evans, 1950), was thus excluded as a factor in the aetiology of the bovine syndrome. Since then, this ethanol extract has been shown to contain a mutagen and a carcinogen; there is considerable evidence indicating that these biological activities are due to the same principle(s). With the aid of a variety of bioassays, (mutagenicity studies using mice and *Drosophila* and the acute and subacute effects following parenteral administration to mice) a toxic chemical has now been isolated and partially characterized by the following procedure.

A hot ethanol concentrate of green dried bracken is poured into excess of cold water and the precipitate discarded. After concentration at low temperature and reduced pressure, the aqueous fraction is treated with charcoal and filtered. The charcoal is eluted with methanol, and the methanol eluate is