have been isolated by using different combinations of selective markers. They are being analyzed further.

With regard to the staining reaction, neither the Hfr nor F+ salmonella give the reaction when mixed with F− strains, either salmonella or coli.

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References

1. L. L. Cavalli, J. Lederberg, E. M. Lederberg, *J. Gen. Microbiol.* **8**, 89 (1953).
2. W. Hayes, *ibid.* **8**, 72 (1953).
3. E. L. Wollman and F. Jacob, *Compt. rend.* **240**, 2449 (1955).
4. R. D. Skaar and A. Garen, *Proc. Natl. Acad. Sci. U.S.* **42**, 619 (1956).
5. E. L. Wollman, F. Jacob, W. Hayes. *Cold Spring Harbor Symposia Quant. Biol.* **21**, 141 (1956).
6. F. Jacob and E. L. Wollman, *Compt. rend.* **240**, 2566 (1956).
7. G. A. Maccacaro, *Nature* **176**, 125 (1955).
8. H. Uetake, T. Nakagawa, T. Akiba, *J. Bacteriol.* **69**, 571 (1955).
9. N. D. Zinder, *Virology* **5**, 291 (1958).
10. T. Loeb, *Science*, this issue.
11. L. S. Baron, W. M. Spillman, N. F. Carey, *ibid.* **130**, 566 (1959).
12. T. Miyake and M. Demerec, *Nature* **183**, 1586 (1959).
13. N. D. Zinder and J. Lederberg, *J. Bacteriol.* **64**, 679 (1952).
14. J. Lederberg, *Methods in Med. Research* **3**, 5 (1950).
15. W. Hayes, *Cold Spring Harbor Symposia Quant. Biol.* **18**, 75 (1950).
16. Y. Hirota, *Proc. Intern. Congr. Genet. 10th Congr.* (1958), p. 121.
17. J. Lederberg and E. M. Lederberg, *J. Bacteriol.* **63**, 399 (1952).

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Suppression of Radiation-Induced Tumorization in Fern Prothalli

Abstract. By quantitative techniques it has been shown that the expected tumor frequency after x-irradiation of spores of the fern *Pteridium aquilinum* can be reduced by the addition of casein hydrolyzate or amino acids to the medium upon which the spores are germinated and grown into either prothalli or tumors.

To analyze the process of tumorization and to attempt to characterize it in terms of fundamental cellular processes, it would seem advantageous to study the phenomenon in the simplest biological system in which it can clearly be recognized. One such system, which is perhaps unique in many ways, is that of the prothallial or gametophytic generation of the fern (*1*). In *Pteridium aquilinum* (the bracken fern) a readily recognizable tumorization occurs spontaneously at a very low rate, but the frequency can be increased in response to ionizing radiations (*2*). Although certain criteria for the word *tumor* are inapplicable here, since *tumor* was originally defined in the frame of reference of more complex biological systems, these abnormal growths are considered to be tumors in that they are unlimited, disorganized growths characterized by many cellular changes typical of tumors in higher organisms. At least in the lower dose range, the response to ionizing radiations is linear, and the spontaneous frequency is increased by approximately 100-fold at 15,000 r of x-rays applied to dormant spores. This process can be quantified quite precisely (*3*). The primary causal event to subsequent tumorization is known to occur in a single haploid cell, the spore. The spores and the resultant prothalli and tumors are germinated and grown aseptically in vitro on chemically defined media.

To study factors involved in tumorization in this system, experiments were performed as follows (*4*): Spores of *Pteridium* in the dormant condition were irradiated at 1000 r/min of unfiltered 136-kv x-rays for a total dose of 15,000 r. The spores were then sterilized and sown either quantitatively (*3*) into petri plates, or semiquantitatively onto agar slants in 25-mm culture tubes. The quantitative technique was used to assay possible effects on viability, whereas the more rapid semiquantitative technique was used for preliminary and large-scale screening. In this latter method, a Carbowax suspension of spores was made as for the fully quantitative method, but with a higher concentration of spores (100 mg/cm^3). Then quantitatively comparable inocula were taken for the control and experimental lots, without making actual spore counts, by dipping a suitable wire inoculating loop into the spore suspension and streaking this onto the slanted agar surfaces. It has been found that the drop which fills the loop contains approximately identical inocula in repeated immersions from one spore suspension.

To assay for the effects of additions to, or alterations of, the medium, these quantitatively similar inocula of x-irradiated spores were streaked onto the control, or basal, medium and onto the experimental media, in rotation. The basal medium is essentially a Knudson's mineral salt solution with added minor elements, sugar and agar (*1*); in all experiments here described spores were grown on 0.25 percent sucrose and 1.5 percent Difco Bacto-agar. This selection of a basal medium was purely arbitrary; it was an outgrowth of earlier work with the culture of fern prothalli. Since the discovery of the phenomenon of induced tumorization and all earlier quantitative work had been on this medium, it was used as a point of departure for subsequent work. Thus, in all experiments the "basal" tumor frequency is defined as the frequency of tumors for each individual experiment on this basal or control lot. Experimental lots are then expressed on a percentile basis as compared with the control lot. The experimental lots have either this basal medium plus an added substance, or, in later experiments, a modification of the basal medium. All recognizable tumors were counted at the end of 6 weeks in culture.

Table 1. Frequency of tumors in prothalli of *Pteridium aquilinum* on various media after x-irradiation of dormant spores, expressed as percentage of frequency on basal medium.

Addition to basal medium	Tumor frequency (%)
Casein hydrolyzate, 2.5 ml/lit. (see *5*)	42
Casein hydrolyzate, 5.0 ml/lit.	34
Synthetic casein hydrolyzate (total)*	34
Monoaminomonocarboxylic amino acids	52
Hydroxyamino amino acids	40
Sulfur-containing amino acids	35
Benzenoid amino acids	42
Basic amino acids	82
Acidic amino acids	96
Pyrrolidyl amino acids	40
NH_4NO_3, 680 mg/lit.	109
NH_4NO_3, 1360 mg/lit.	116

* Our synthetic approximation of casein hydrolyzate, compiled from amino acids obtained from Mann Research Laboratories, Inc., New York, was made up as follows, in milligrams per 100 ml of stock solution, used at the rate of 100 ml/lit. of medium: monoaminomonocarboxylic: glycine (20), L-alanine (35), L-valine (70), L-leucine (100), and L-isoleucine (60); hydroxyamino: L-serine (60) and L-threonine (45); sulfur-containing: L-cystine (5) and L-methionine (30); benzenoid: L-phenylalanine (50), L-tyrosine (60), and L-tryptophane (20); basic: L-lysine (70), L-arginine monohydrochloride (50) and L-histidine monohydrochloride (30); acidic: L-aspartic acid (70) and L-glutamic acid (220); pyrrolidyl: L-proline (80) and L-hydroxyproline (20).

With this experimental approach, first various plant growth hormones (auxins) were tested, but with no apparent effect. This emphasized the possible magnitude of the task if future testing were to be done with specific substances. Thus, in an attempt to test broader categories of substances, casein hydrolyzate (*5*) was among those tested. This was found to depress the incidence to as low as 34 percent of the basal rate in the concentrations used. For further information on this and other results discussed, see Table 1. Several replications confirmed this initial observation. Next, to determine whether this effect was due to the amino acids, a synthetic approximation of casein hydrolyzate was made, based upon published analyses of casein (*6*). This synthetic "casein hydrolyzate" was found to have a similar tumor-inhibiting effect, depressing the incidence to the lowest value observed with the original hydrolyzate. Next, the component amino acids were arbitrarily categorized as to structural types, and these groups were tested separately, in the same concentrations in which they occurred in the total mixture. The effectiveness of the groups varied, and no one group was quite as effective as the total, but the results indicated that the effectiveness of the total mixture was due to several of the component amino acids, and also that the action of the various amino acids was more

than additive. That is, the separate groups tested seemed to be more effective than would have been expected. The basic and acidic amino acids were not significantly effective, whereas all other groups had marked tumor-depressing activity. These results suggested that although there may be some sort of specificity with respect to certain amino acids or types of amino acids, there also appeared to be nonspecificity in that suitable amino acids could satisfy most of an apparently general requirement.

To test the effect of simply raising the nitrogen level of the medium, the addition of ammonium nitrate to the basal medium in concentrations sufficient to double and triple the nitrogen supply showed instead a very slight increase in tumor frequency. To establish that the decrease in tumor frequency produced by the casein hydrolyzate or the amino acid mixture was not simply due to a comparable decrease in general viability, the fully quantitative method was used to determine the viability in each treatment. Although the two additions to the media did depress viability slightly, this was not in proportion to the decrease in tumor counts. The tumor frequencies, when adjusted to percentage viability were: casein hydrolyzate, 57 percent; synthetic "casein hydrolyzate," 47 percent, the basal frequency being expressed as 100 percent. The viability on the casein hydrolyzate was 93.5 percent of that on the basal medium, and on the synthetic "casein hydrolyzate" was 91 percent of the basal value.

It is apparent from these results that it is possible in this system to influence the frequency of occurrence of tumors. Of course, there is no ready way to determine at present whether the potentially tumorous spores do germinate on the amino acid-containing media. They could either not develop at all, or alternatively, they could develop into normal prothalli. The latter case could mean that in the potential tumors there is a block in the pathway from inorganic nitrogen to $-NH_2$ and that the amino acids are in effect satisfying a continuing deficiency, or possibly that the amino acids may be necessary just at the start of physiological activity, perhaps to repair radiation damage (*7*). At present, experiments are being devised to attempt to establish the time relationship of the phenomenon. Since the observation of the amino acid effect, investigations on inorganic nitrogen metabolism have also been initiated. Present results show that with ammonium as the sole source of nitrogen, tumor frequency is highest, whereas with nitrate as the sole source, the frequency is lowest, viability on calcium nitrate being 93.5 percent of that on a nitrogen equivalent of ammonium sulfate. These results would suggest further a possible block in nitrogen reduction from $-NO_3$ to $-NH_4$ associated with the initiation of tumors.

That only a fraction of the total expected number of tumors is affected by any one treatment would suggest that what we recognize as the morphological entity "tumor" actually represents a whole spectrum of biochemical variants. Conceivably, these variants may fall into more general categories in that they affect certain general mechanisms, the dysfunctions of which may also have the general morphogenetic manifestation of tumorization.

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References and Notes

1. T. A. Steeves *et al.*, *Am. J. Botany* **42**, 232 (195; C. R. Partanen *et al.*, *ibid.* **42**, 245 (1955); C. R. Partanen, *Cancer Research* **16**, 300 (1956).
2. C. R. Partanen and T. A. Steeves, *Proc. Natl. Acad. Sci. U.S.* **42**, 906 (1956).
3. C. R. Partanen, *Science* **128**, 1006 (1958).
4. This investigation was supported in part by a grant (No. CY3335) from the National Institutes of Health, U.S. Public Health Service. I wish to thank the Harvard Biological Laboratories for the use of their facilities during a part of these investigations. I am indebted to G. S. Lord for having made the spore collections, and to Miss Jane Nelson for her technical assistance.
5. "Vitamin free" casein hydrolyzate-acid, Nutritional Biochemicals Corp., Cleveland, Ohio.
6. R. J. Block and D. Bolling, *The Amino Acid Composition of Proteins and Foods* (Thomas, Springfield, Ill., ed. 2, 1950).
7. R. F. Kimball *et. al.*, *Proc. Natl. Acad. Sci. U.S.* **45**, 833 (1959).

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Isolation from Animals of Human Strains of Staphylococci during an Epidemic in a Veterinary School

Abstract. Antibiotic-resistant staphylococci of the same bacteriophage type were isolated from the external nares of asymptomatic domestic animals and from human beings during an epidemic of staphylococcal disease in veterinary students. Not all of the infections could be attributed to person-to-person contact; spread could be explained also by transmission of staphylococci from animals.

An unusual number of senior students at the Veterinary School of the University of Pennsylvania reported to the student health service during the school year 1956–57 with furuncles, cellulitis, deep cutaneous abscesses, and paronychiae. A high incidence of infections continued for the next 2 years, and contact infections in students' families were also observed. Cultures obtained from the lesions and from the external nares of the students yielded hemolytic coagulase-positive *Staphylococcus aureus* of the bacteriophage type 52/42B/81 (type 80/81). The strain was resistant to penicillin, streptomycin, and the tetracyclines and was occasionally resistant to erythromycin; it was sensitive to chloramphenicol and novobiocin.

The external nares of both animals and human beings were swabbed with dry cotton-tipped sticks, which were then rolled on blood-agar plates or, in the third animal survey, on saline (7.5 percent)–mannitol (1 percent) plates, and streaked within 1 hour of swabbing. After incubation overnight, colonies were selected without regard to pigmentation and subjected to the tube-coagulase test with human serum. All coagulase-positive or mannitol-fermenting strains were tested with 23 standard bacteriophages at the routine test dilution (*1*).

In May 1957 questionnaires were distributed to determine the staphylococcal morbidity in the school (Table 1). Thirty-six percent of the seniors reported having had lesions during the school year. The faculty also reported a high incidence (26 percent), but the other three classes of students reported an incidence less than half that of the seniors. Ten of the 11 active lesions cultured yielded staphylococci of strain 80/81.

Nasal swabbing was also performed in May 1957 (Table 1); 46 percent of the seniors carried strain 80/81 staphylococci, whereas the carrier rate in the other five groups was 14 percent or less. Two of the four employees who were carriers were stablemen, and two were office workers. The two faculty carriers and other faculty members who later became carriers all worked in the animal clinics.

During the next school year (1957–58) nasal swabbing of the 46 new seniors was repeated regularly to determine the source of infection. Five seniors were carriers of the 80/81 strain in September 1957; only one of these had been a carrier as a junior. The other four students had worked in the school during the summer. Ten other seniors became carriers before the end of the school year. Lesions developed in all but two of these 15 students. All six of the lesions cultured yielded 80/81 staphylococci. Analysis of the dates on which students were first shown to be carriers revealed that they became carriers shortly after they had been in a work group which included a student with an active lesion.

A second morbidity survey and program of nasal swabbing of the students during the school year 1958–59 (Table 1) showed, as the first had, that the current seniors most frequently gave a history of a recently acquired lesion and most frequently carried the 80/81 strain. In February 1959 no isolation