absolute darkness. No toxicity was ever observed in hematoporphyrin solutions in 1:20,000 in semidarkness; indeed, paramecia multiplied in such concentrations.

In the preliminary experiments, it was found that hematoporphyrin altered the radiation sensitivity in such a pronounced manner that it was difficult to find a radiation dosage that did not kill all the paramecia. For example, a dose of 85 kr killed all the animals in porphyrin solutions of 1:10,000 to 1:40,000 hematoporphyrin but not those in irradiated nonporphyrin control solutions. In another experiment using 1:20,000 hematoporphyrin solutions, 18 percent of the animals were killed with 34 kr and 90 percent were killed with a dose of 51 kr, whereas all the paramecia not treated with porphyrin survived a dose of 100 kr.

An examination of the data from later experiments revealed that all of the control paramecia, not treated with porphyrin, survived a dose of 100 kr, and that some died with greater dosages. In the lower concentrations of porphyrin of 1:200,000 and 1:500,000, all paramecia survived dosages up to 80 kr, but some died with higher dosages. It was noted that with higher concentrations of porphyrin, such as 1:20,000 to 1:100,000, all the animals survived only the relatively small doses of 5 to 20 kr, respectively.

Our results have been summarized in the form of two survival curves (Fig. 1). A comparison of the two curves indicates in a striking manner that hematoporphyrin sensitizes paramecia to x-radiation. The LD-50, 24 hr, of the control paramecia was 340,000 r, whereas the LD-50 of porphyrin-treated paramecia was only 18,000 r.

The possibility that lethality may have resulted from summation of toxic effects of hematoporphyrin and radiation was considered but was ruled out for several reasons. The effective concentrations of hematoporphyrin are not toxic, even when they are applied to the paramecia for 8 or 10 days. Moreover, the sensitizing influence varied directly with the concentration, and the dose of x-radiation that killed all or most paramecia in the presence of porphyrin (5 to 25 kr) is small when it is compared with the minimum lethal dose (100 kr) for control paramecia.

An additional observation made possible by the plastic-syringe method (*5*) is pertinent and of importance in other respects. The paramecia in the plastic syringes in porphyrin solutions were not killed by the relatively low doses of 20 to 80 kr, as long as they were retained in the syringe. When they were expelled and exposed to air in depression slides, they died within a period of 20 to 45 min. In one experiment, this feature was studied in some detail. After the necessary samples for counting and observation had been ejected into observation dishes, the remainder were left in the syringe to see how long they would survive in the syringe. The paramecia in a 1:20,000 solution of hematoporphyrin that had received a dose of 80 kr were still alive 22 hr later, but when they were ejected into observation dishes and exposed to air, they died within 45 min.

Thus, the sensitization of paramecia to radiation by hematoporphyrin is not a summation of toxic effects but an oxygen-dependent sensitization. The fact that oxygen is required and that it may be effective when it is added to the set of factors several hours after the period of irradiation is of greater interest and will be studied more extensively.

The hypothesis is therefore presented that one of the primary reasons for the increased x-radiation susceptibility of dividing cells is that they contain higher concentrations of porphyrin than do differentiated nondividing cells. In animals with transplanted tumors and in human cancer patients, the injection of hematoporphyrin causes the tumor tissue to accumulate even more porphyrin (*1, 7*). The hope that the efficiency of radiation therapy of cancer may be improved by injections of hematoporphyrin is thus strengthened by these observations, which demonstrate in a conclusive manner that this chemical renders single cells, such as *Paramecium,* much more susceptible to the lethal effects of x-radiation.

FRANK H. J. FIGGE
Department of Anatomy, University of Maryland Medical School, Baltimore
RALPH WICHTERMAN
Department of Biology, Temple University, Philadelphia, Pennsylvania

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10 February 1955

Mucunain, the Active Pruritogenic Proteinase of Cowhage

Cowhage is the name given to the short barbed hairs (trichomes) that cover the seed pods of the tropical plant *Mucuna pruriens.* An exquisitely effective stimulus for itching, cowhage has long been known as itch powder. This report (*1*) outlines our technique in extracting, isolating, and identifying the active pruritogenic principle of cowhage.

Untreated cowhage was extracted for 5 min with 0.9-percent aqueous sodium chloride solution (0.8 ml/100 mg). After vacuum filtration (Schleicher and Schuell, No. 602), 2 vol of absolute ethyl alcohol was added slowly while the extract was stirred. The resultant flocculent precipitate was removed 10 min later by centrifugation (1700 rev/min, 3 min), rapidly air-dried, and redissolved in 0.9-percent sodium chloride solution. Reprecipitation with methyl alcohol (2 vol) was done, and the final product was redissolved in half the volume of sodium chloride solution.

It was necessary to carry out all procedures at 0°C and to pay careful attention to reagent purity and glassware cleanliness. At all stages of isolation, activity checks were run by injecting 0.02 ml of solution intradermally. All fully active preparations produced an intense localized itch, which appeared after 5 to 10 sec and persisted for 3 to 5 min. This itch was of an entirely different order of magnitude than that experienced following electric stimulation or the injection of histamine or histamine liberators. It was accompanied by slight to moderate local whealing.

Study revealed that the "itch substance" was nondialyzable and extremely labile, suggesting a protein nature. Changes in *p*H, heating, or any simple denaturant process rapidly inactivated the extract. However, activity of any extract could be preserved for months by rapid drying at low temperatures or by freezing. Biological and chemical tests made for numerous pharmacologic agents led to the conclusion that an enzyme was responsible for the itch properties of cowhage. Following this, *amylase* was demonstrated in the initial aqueous extract of the *Mucuna* spicules but was shown to be present in fractions other than the purified pruritogenic one. Finally a broad spectrum proteinase was discovered to be present in all the active pruritogenic extracts and preparations. Furthermore, its activity was correlated with pruritus but not with urticaria. This proteinase, hitherto undescribed, has been named by us *Mucunain* to indicate its source and nature.

Mucunain attacks the following synthetic substrates (0.01*M* substrate, 60-min incubation at 35°C, *p*H 5 to 8,

M/15 Na. K phosphate buffer, 25-percent aqueous phenol ascending Whatman No. 1 paper chromatogram, ninhydrin): carbobenzoxy-glycyl-l-phenylalanine, carbobenzoxy-glycyl-l-phenylalanine amide, and carbobenzoxy-glutamyl-l-tyrosine. Under these conditions no activity could be shown against benzoyl argininamide, benzoyl glycinamide, benzoyl arginine methylester hydrochloride, or leucyl glycylglycine. In common with all proteinases, mucunain has the property of coagulating milk. It is neither activated nor inactivated by cysteine. No coenzyme or cofactor is known to be necessary.

Although Broadbent (*2*) claimed to have made an active extract of cowhage by boiling, we have been unable to confirm his work. Boiling destroys mucunain instantaneously. Furthermore, we have found that serotonin (5-hydroxytryptamine), which has recently been identified in cowhage (*3*), is without pruritogenic properties.

WALTER B. SHELLEY
ROBERT P. ARTHUR
Department of Dermatology,
University of Pennsylvania
Medical School, Philadelphia

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12 January 1955

Metabolism of 3,4-Benzopyrene

In following up the earlier work of Chalmers and Peacock (*1*) Weigert and Mottram (*2*) demonstrated, in the excreta of mice injected with 3,4-benzopyrene, the presence of four distinguishable metabolites, X_1, X_2, F_1 and F_2. Of these, F_2 corresponded in its absorption spectrum and phenolic properties to the metabolic phenol 8-benzopyrenol, previously identified by Berenblum and Schoental (*3, 4*) and subsequently confirmed by synthesis (*5*). By analogy with anthracene, in which metabolic oxidation had been shown to undergo perhydroxylation with the formation of a diol (*6*), Weigert and Mottram (*2*) postulated the schematic metabolic pathway for 3,4-benzopyrene that is shown.

In fact, the nature of the metabolites X_1 and X_2 has not yet been established, and the suggestion that these represent derivatives of 8,9-dihydrodihydroxybenzopyrene does not seem justified. For instance, the long-wave systems of the absorption spectra of these two products (as reproduced in the publications of Weigert and Mottram) are very similar to those of fully aromatic benzopyrene derivatives, with their longest absorption bands *displaced to the red* in relation to those of benzopyrene, which is contrary to what one would expect if the 8,9 positions were hydrogenated. The assumption that F_1 is a conjugated 8-benzopyrenol is also unjustified, for its adsorption properties are not very different from those of F_2, while the fluorescence spectral bands, on the column, are displaced by about 7 mμ to the ultraviolet in comparison with those of F_2. In all these respects, F_1 appears to be indistinguishable from unconjugated 10-benzopyrenol (*4*), rather than a conjugated 8-benzopyrenol.

It remains to be shown whether the metabolites X_1 and X_2 represent respective conjugated products of the phenolic 10- and 8-benzopyrenols, and if so, what the nature of the conjugation compounds is. But the scheme of Weigert and Mottram, as it stands, not only lacks confirmation but is actually inconsistent with the known facts.

This note was prompted by the perpetuation in recent reviews and other works of reference (*7*) of Weigert's scheme, which, in the light of subsequent evidence, especially about the existence of 10-benzopyrenol and its probable identity with F_1, is no longer acceptable. Were it not for his untimely death, Weigert would no doubt have corrected the scheme himself.

I. BERENBLUM
Department of Experimental Biology,
Weizmann Institute of Science,
Rehovoth, Israel

R. SCHOENTAL
Cancer Research Department,
Royal Beatson Memorial Hospital,
Glasgow, Scotland

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Attempt to Reproduce Some of Moewus' Experiments on Chlamydomonas and Polytoma

During the stay of Franz Moewus and his wife in this laboratory, sympathetic and conscientious efforts were made by members of our group to repeat a number of his experiments. These involved monoecious and dioecious strains of *Chlamydomonas eugametos,* some mutants of the latter, and a monoecious strain of *Polytoma uvella.* Culture fluids of male and female *C. eugametos* and the compounds phenylalanine, rutin, isorhamnetin, and paeonin were utilized; for all of these, specific activities had been reported. The material and conditions used were those of Moewus, the experiments were of his choice, and he participated in some of them. Since, after 16 months no substantial confirmation of his claims is at hand, even for experiments originally performed by Moewus and his wife in this laboratory [F. Moewus, *Biol. Bull.* **107,** 293 (1954)], our attempts at repetition have been discontinued. A mimeographed description of the experiments that were done can be obtained from the present author.

FRANCIS J. RYAN
Department of Zoology,
Columbia University, New York

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