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.

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References and Notes

1. This study was supported by Army contract DA-49-007-MD-154.
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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.

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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.

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