

Further experiments with erythrocytes from *P. berghei*-infected mice and from *P. vinckei*-infected hamsters have revealed no significant increases in the levels of these enzymes on infection, but the erythrocytes of both animals before infection have much higher enzyme levels than those of uninfected monkeys. There was no evidence of any reticulocytosis in either of these infections.

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Isolation of an Anabolic, Uterotrophic Compound from Corn infected with *Gibberella zeae*

A METABOLITE with possible anabolic and marked uterotrophic activity has been isolated from extracts of the fungus *Gibberella zeae* (*Fusarium graminearum*).

Numerous reports of genital involvement in animals fed spoiled grain¹⁻⁴ prompted us to investigate the possible relationship of micro-organisms to this syndrome. During 1957 and 1958 we observed seven widely separated swine herds showing vulvar hypertrophy and occasional vaginal eversion in females, preputial enlargement in castrated males and prominent mammary glands in both sexes. In each case the animals were fed moulded corn. Spoiled grain was procured from each farm, treated with sodium hypochlorite and plated on potato-dextrose agar. Predominant micro-organisms isolated from the samples were *Penicillium* spp., *Cladosporium* sp., *Mucor* spp. and *Gibberella zeae*. Cultures of these moulds were grown on sterile ground corn (35 per cent moisture) in flasks stored at about 24° C for 2-3 weeks. This moulded corn was fed *ad libitum* to sexually immature female pigs. Within four days, vulvar and mammary enlargement were elicited only in those animals fed corn seeded with *G. zeae* isolates. Similarly prepared corn fed for six days to five mature, ovariectomized mice produced an average uterine weight of 94.3 mg compared with 22.6 mg for the control animals. An alcoholic extract of the *Gibberella* moulded corn incorporated in sesame oil and injected subcutaneously into 10 castrated female mice at a dose of 0.1 ml. per animal daily for 3 days produced an average uterine weight of 60.7 mg compared with 20.6 mg for the controls. Nine isolates of *G. zeae* were obtained which produced varying degrees of uterotrophic activity.

To obtain material for chemical and pharmacological tests, a study was made of the cultural requisites for the production of the active principle. Suitable

substrates include wheat or barley as well as corn or a synthetic medium containing mineral salts and carbohydrates including corn starch, high test molasses or D-glucose. An easily separable, water-soluble fraction, toxic for mice, is occasionally produced concurrently with the metabolite. This confirms the earlier work of Christiansen and Kernkamp⁵ and Mitchell and Beadles⁶ associating toxicity with the ingestion of *G. zeae*-infected grain.

Recovery of the metabolite is effected by water extraction of the corn-mycelial mixture in a high-speed blender to remove the water-soluble fractions. The wet cake remaining after filtration is repeatedly blender-extracted with anhydrous ethanol to remove the active principle. The alcoholic extracts are combined and the solvent removed by distillation. An aqueous-organic phase partition of the resulting residue is followed by application of the concentrate to a column of synthetic magnesium-silicate. Subsequent development by gradient elution using solvents of increasing polarity separates the active component. The solvent mixture is evaporated from the active fraction resulting in a white, crystalline mass which upon recrystallization has a melting point range of 161°-163° C, (α)₂₅^o/Hg = -82° (c. = 2.4 ethanol). A methanolic solution of the metabolite exhibits a bright, greenish-blue fluorescence when exposed to ultra-violet light. Ultra-violet absorption peaks occur at about 237, 275 and 315 m μ (methanol). Additional chemical, physical and structural data will be forthcoming as pure material becomes available.

Preliminary experiments indicate that partially purified concentrates of the active principle are effective in improving growth-rate and feed efficiency in sheep.

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Bile Pigment in *Eucypris virens* (Jurine)

Eucypris virens (Crustacea : Ostracoda) is a common ostracod with green patches on the valves of its carapace. When treated with yellow nitric acid these green patches become purple, then red, orange and finally yellow. This sequence is typical of the later stages of the Gmelin reaction, indicating the presence of a bile pigment.

Several hundred *E. virens* were soaked overnight in acid methanol (5 per cent hydrochloric acid), to give a clear blue-green solution with an absorption maximum at 655 m μ . The pigment passed readily into chloroform after dilution of the acid methanol with water. In dry chloroform absorption maxima were found at 362 and 660 m μ . The chloroform solution was then evaporated and the pigment taken into methanol. The solution in methanol did not fluoresce