P. viridicatum strains showed a radiation damage of 34% at 0.06 and 41% at 0.08 Mrad (Figure 1) during the first 5 days; recovery set in after this period. On the tenth day *P. viridicatum* strains at both doses showed an average recovery of about 28%. No conspicuous further recovery was observed on or after the twelfth day. In contrast, however, *A. flavus* strains had a negligible damage by the same radiation doses. Age of the fungi strains plays an effective role in the susceptibility to β-radiation, i.e. conidia of the strains of 6-month-old *A. flavus* and *P. viridicatum* cultures were more susceptible to irradiation than 3-week-old (Figures 1 and 2).

A difference in the susceptibility among strains of *P. viridicatum* as well as of *A. flavus* was noted. One strain (No. 128) of the former showed no growth at all at 0.08 Mrad and the other (No. 129) showed no conidial growth both at 0.06 and 0.08 Mrad. Of *A. flavus* strains the most susceptible was No. 376. Regardless of the age of fungal species and strains studied, *P. viridicatum* and *A. flavus* growth was inhibited when irradiated with 0.2 Mrad and above. Of 195 inoculation points in petri dishes irradiated at 0.2 Mrad, and another 195 irradiated at 0.5 Mrad, not a single one showed any growth. In general *P. viridicatum* strains were more sensitive to β-radiation than those of *A. flavus*. Figure 3, a and b clearly show the inhibiting effect of irradiation on mycelial and conidial growth. Studies are now in progress to correlate differential radiation sensitivity with differences in mycelial and conidial cell wall structure[6].

Zusammenfassung. Die β-Strahlen-Empfindlichkeit von 7 *P.-viridicatum*-Stämmen und 6 *A.-flavus*-Stämmen wurde an Kulturen verschiedenen Alters untersucht. Eine Dosis von 0,2 Mrad verhinderte das Wachstum 3 Wochen alter Kulturen beider Gattungen vollständig, während bei 6 Monate alten Kulturen eine Dosis von 0,1 Mrad genügte.

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[6] The excellent technical assistance of Mrs. Zimmermann and Miss Runge is acknowledged.

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4-Methylcatechol, a Metabolite of Homoprotocatechuic Acid

The metabolism of homoprotocatechuic acid (3,4-dihydroxyphenylacetic acid) in rats and rabbits has been studied by Booth et al.[1,2] and Scheline, Williams and Wit[3]. The latter authors, using (carboxy-^{14}C) homoprotocatechuic acid given to rabbits orally at a dose level of 100 mg/kg, found that all of the administered radioactivity could be recovered in the urine after 8–9 days. The radioactivity in the 44 h urines amounted to about 85% of the dose and was found in the following metabolites: homoprotocatechuic acid (63%), homovanillic acid (5.6%), *m*-hydroxyphenylacetic acid (14%) and *p*-hydroxyphenylacetic acid (1.4%) of the dose. Similar experiments using 3 rats (Scheline, Williams and Wit, unpublished results) showed that the 44 h urines contained about 80% of the radioactivity which was found in the above metabolites to the extent of 55, 18.5, 6.5 and 1.4% of the dose, respectively. The mean recovery of radioactivity in the urine and faeces was 93% after 13 days.

The dehydroxylation of catechol acids has been suggested to be a reaction which is carried out by the microflora of the intestinal tract (Shaw et al.[4]). Booth and Williams[5,6] reported that several catechol acids, including homoprotocatechuic acid, are converted to *m*-hydroxy derivatives by rat faecal and caecal contents, and Perez-Silva et al.[7] have recently isolated a strain of Psuedomonas *sp.* from rat faeces which could dehydroxylate caffeic acid (3,4-dihydroxycinnamic acid). As part of a study to investigate the metabolic capabilities of the gastrointestinal flora towards foreign organic compounds, the dehydroxylation reaction was studied according to the method described previously[8,9]. When homoprotocatechuic acid was incubated anaerobically with rat faecal extracts, *m*-hydroxyphenylacetic acid was observed chromatographically in 7 of 9 experiments. In addition, a prominent spot was observed on all the chromatograms which was not seen when homoprotocatechuic acid was incubated with the medium alone or with faecal contents and oxytetracycline (8 μg/ml). This substance was also formed when rat caecal contents were used. A 0.01 *N* HCl solution of this substance obtained from a thin-layer chromatogram showed an absorption maximum at 279 nm (Beckman DB) and a fluorescence maximum at 323 nm (Aminco-Bowman Spectrophotofluorometer, uncorrected value). These values suggested that the substance was a catechol and its Rf value in benzene-glacial acetic acid-water (6:7:3) (BAW) suggested that it was a catechol slightly less polar than pyrocatechol.

It has been found that a number of *p*-hydroxybenzoic acid derivatives, including protocatechuic acid, are decarboxylated to the corresponding phenols by rat faecal and caecal contents[8]. A similar decarboxylation of the phenylacetic acid derivatives would lead to the 4-methylphenols and, in the case of homoprotocatechuic acid, to

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4-methylcatechol. Thin-layer chromatograms on cellulose showed that the unknown and 4-methylcatechol gave the same Rf values in BAW and 20% KCl-glacial acetic acid (100:1) (KClA) and both compounds produced a grey-violet colour when sprayed with fast blue B salt followed by saturated sodium bicarbonate solution. A solution of 4-methylcatechol in 0.01 *N* HCl gave the same UV-absorption and fluorescence maxima as those obtained with the unknown. Further confirmation was obtained by comparing the IR-spectra (KBr disc) of the metabolite and 4-methylcatechol following their isolation from paper chromatograms developed in BAW.

To study the possible significance of these findings for the metabolism of homoprotocatechuic acid in animals, 6 white rats (males, 320–340 g) were each given an aqueous solution containing 100 mg of this substance by stomach tube. The 24 h urines were collected in containers placed in solid carbon dioxide and, after acid hydrolysis and ether extraction, were examined by thin-layer chromatography in the above solvent systems. All of the chromatograms showed prominent areas corresponding to 4-methylcatechol. The chromatograms also showed the presence of homoprotocatechuic acid and its previously reported metabolites, except for *p*-hydroxyphenylacetic acid which is obscured under these chromatographic conditions. Isolation of the urinary 4-methylcatechol was accomplished by paper chromatography with BAW and then KClA. The UV-absorption and fluorescence maxima of the substance eluted with 0.01 *N* HCl were identical with those of 4-methylcatechol. The IR-spectrum of the isolated material confirmed these findings. Qualitatively similar results were obtained when a dose level of 100 mg/kg was used (3 rats). 4-Methylcatechol was not observed on the chromatograms of similarly treated urines from 3 control rats, nor was it found in the urines when homoprotocatechuic acid (100 mg) was given to 2 rats by i.p. injection. Homoprotocatechuic acid is stable under the conditions of hydrolysis used and no 4-methylcatechol was seen on the chromatograms when 100 mg of homoprotocatechuic acid was added to normal urine before hydrolysis.

Work is presently in progress which aims to extend the study to include other C_6-C_2 as well as C_6-C_3 phenolic acids. Some preliminary results indicate that these substances may undergo demethylation and reduction of double bonds, as well as dehydroxylation and decarboxylation, when incubated with extracts of rat caecal contents or faeces. These findings suggest that the intestinal microflora may be of considerable significance in determining the metabolic fate of plant phenolics[10].

Zusammenfassung. Homoprotocatechusäure wird in der Ratte bei oraler Zufuhr teilweise durch Dekarboxylierung zu 4-Methylcatechol abgebaut, eine Reaktion, die von oxytetracyclinsensitiven Darmbakterien ausgeführt werden kann.

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Atypical Mitochondrial Morphology of the Intestinal Absorptive Cells of the Germfree Rat

In recent years, the absorption mechanisms of normal intestinal mucous membrane have been investigated by electron microscopy by several workers[1–4]. Our investigation was prompted by the reported differences between the gastrointestinal tracts of germfree and open animal room guinea-pigs[5], as judged by light microscopy, and the differences in fecal nitrogen excretion by germfree and conventional rats[6]. We found a striking variation in mitochondrial morphology in germfree[7] Fischer rats.

Specimens of duodenum, jejunum, ileum and colon from rats 8 weeks old were prepared for electron microscopy by conventional techniques[9] as well as for light microscopy. The ultrastructure of the absorptive cells in the germfree rats was similar to that of their conventionalized[10] littermates except for the mitochondria. In 3 of the 7 germfree rats examined, a number of absorptive cells, particularly among those located toward the tips of the villi, contained 2 forms of mitochondria which were not found in any of the conventionalized rats. The first type (Figure 2) was round or elongated with one or more deep indentations, which often were wedgeshaped, and clearly bordered by the usual mitochondrial membrane. These indentations contained cytoplasmic ground substance, which was lighter than the rest of the cytoplasm and consisted of very fine granular material with varied electron density and filamentous structures measuring 30–70 Å in width. Examination at higher magnification suggested the presence of a faint microvesicular pattern within these indentations. A similar appearance was

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[10] Conventionalized rats are germfree until weaning at 21 days and then purposefully contaminated with cecal contents from open animal room rats of the same strain. Thereafter, the purposefully contaminated rats are maintained in the same type of plastic isolators, receive the same autoclaved diet, and handling as their germfree littermates.