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.

R. R. SCHELINE

Department of Pharmacology. University of Bergen, Bergen (Norway), 15th December 1966.

[10] The author wishes to thank Mrs. ELI TEPSTAD for technical assistance.

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

[1] R. R. CARDELL JR., S. BADENHAUSEN and K. R. PORTER, Electron Microsc. *2*, 587 (1966).
[2] S. L. PALAY and L. KARLIN, J. biophys. biochem. Cytol. *5*, 363 (1959).
[3] S. L. PALAY and L. KARLIN, J. biophys. biochem. Cytol. *5*, 373 (1959).
[4] E. YAMADA, 16th Nippon Med. Congress *7*, 111 (1964).
[5] H. SPRINZ, D. W. KUNDEL, G. J. DAMMIN, R. E. HOROWITZ, H. SCHNEIDER and S. E. FORMAL, Am. J. Path. *39*, 681 (1962).
[6] S. M. LEVENSON and B. TENNANT, Fedn Proc. Fedn Am. Socs exp. Biol. *22*, 109 (1963).
[7] The term germfree as used in this paper refers to rats free of viable bacteria, parasites and fungi as determined by methods described elsewhere[8]. The rats were housed in flexible plastic film isolators.
[8] S. M. LEVENSON, R. P. Mason, T. E. HUBER, O. J. MALM, R. E. HOROWITZ and A. EINHEBER, Ann. Surg. *150*, 713 (1959).
[9] J. H. LUFT, J. biophys. biochem. Cytol. *9*, 409 (1961).
[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.

usually noted in both the inner and outer mitochondrial matrices. In some instances, the mitochondrial membranes along the indentation revealed a distinct separation of the inner and outer membranes, creating a wide space or compartment, and in others, the outer mitochondrial membrane showed an interruption along the indentation (Figure 2 arrows). These atypical mitochondria were often very close to the granular endoplasmic reticulum. In favorable sections, the agranular endoplasmic reticulum was always closely applied at the site of indentation and often appeared to terminate with the open end directed into these indentations.

The second form of atypical mitochondria encountered in germfree rats was also seen exclusively towards the tip of the villi. The mitochondria of the second form were again mostly round to elongated in shape, but without the indentation characteristic of the first type. The intramitochondrial granules were not prominent. The outer membrane of the mitochondria seemed to be well defined, but the inner matrix consisted of many irregular vesicular profiles, derived seemingly from dilated mitochondrial cristae. The membrane of these vesicular cristae showed short angular segments. The surrounding cytoplasmic matrix consisted chiefly of agranular and granular vesicles.

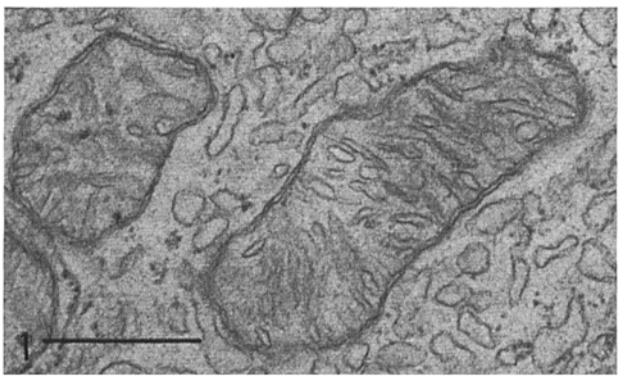
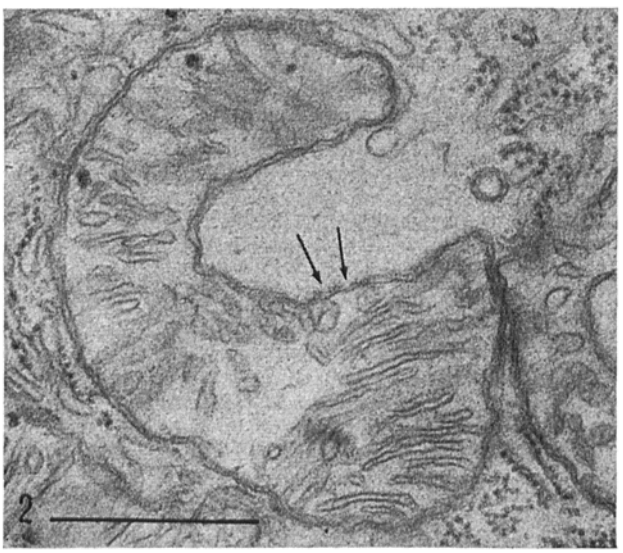
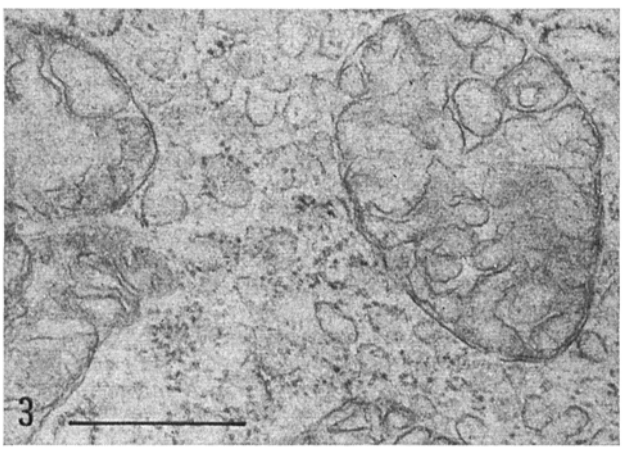


Fig. 1. Mitochondria in an intestinal absorptive cell of a conventionalized rat.

Both types of atypical mitochondria were abundant and were mixed among the usual type of mitochondria (Figure 1) seen in the intestinal absorptive cells of the germfree rats. They were localized strictly between the Golgi field and the terminal web. The morphological variations of mitochondria were not attributable to mobility of the mitochondria or to the direction of sectioning. This particular type of mitochondrial atypism has not been reported elsewhere. Reports of 'unusual' shaped mitochondria in tissues of ordinary open room laboratory animals suggest that they are frequent in endocrine organs[11–15], but none of those reported are like those seen in the germfree rats. Recently, STEPHENS and BILS[16] have reported cupshaped mitochondria in the normal rat liver. Possible physiological significance of morphological changes in the mitochondria has been discussed by PALADE and SCHIDLOWSKY[17]. They originally described a lipid mass closely surrounded by ringform mitochondria; the lipid mass was seemingly in contact with the inner mitochondrial membrane. They attributed the close association of lipid and mitochondria as representing a shift from carbohydrates to lipids as a main source of cellular energy. LEHNINGER[18] speculated that 'local gradients or discontinuities in concentration of critical factors could cause localized swelling or contraction of portions of the surface of single mitochondria'. He also mentioned the possibility that fatty acids could cause local swelling of the mitochondria at the point of contact with the endoplasmic reticulum. This suggestion is based on the work of AVI-DOR, who found a fatty acid subfraction of microsomes which caused swelling of rat liver

[11] A. K. CHRISTENSEN and G. B. CHAPMAN, Expl Cell Res. *18*, 576 (1959).
[12] B. L. MUNGER, Am. J. Anat. *103*, 275 (1958).
[13] E. DEROBERTIS and D. D. SABATINI, J. biophys, biochem. Cytol. *4*, 667 (1958).
[14] T. N. TAHMISIAN, E. L. POWERS and R. L. DEVINE, J. biophys. biochem. Cytol. *2*, Suppl. 4, 325 (1956).
[15] D. D. SABATINI, E. D. P. DEROBERTIS and H. B. BLEICHMER, Endocrinology *70*, 390 (1962).
[16] R. J. STEPHENS and R. F. BILS, J. Cell Biol. *24*, 500 (1965).
[17] G. E. PALADE and G. SCHIDLOWSKY, Anat. Rec. *130*, 352 (1958).
[18] A. L. LEHNINGER, Physiol. Rev. *42*, 467 (1962).





Figs. 2 and 3. Atypical mitochondria in an intestinal absorptive cell of a germfree rat. Arrows in Figure 2 indicate an alteration of the external mitochondrial membrane along the indentation (Type 1). Figure 3 shows Type II. The scale indicates ½ μ.

mitochondria[19]. Such a possibility has also been critically discussed by SIEKEVITZ and PALADE[20]. As mentioned in our description, the localization of these mitochondria with altered morphology in the absorptive intestinal cells of germfree rats suggests a relationship to the surrounding structures, especially those just beneath the terminal web where freshly absorbed materials first come into contact with the cytoplasm to be metabolized[1]. The limited biochemical data on germfree rats available does not permit us to draw now any definite interpretation of the changes in mitochondrial morphology. Consideration should be given to their possible relation to cell necrosis, but the type of mitochondrial swelling is different and none of the other changes related to cell necrosis[21] were encountered in these cells[22].

Zusammenfassung. Die Mitochondrien der Dünndarm-Epithelzellen 3 Monate alter keimfreier Ratten zeigten elektronenmikroskopisch auffallende Formunterschiede im Gegensatz zu Vergleichstieren desselben Wurfes, die mit Coecum-Inhalt gewöhnlicher Ratten kontaminiert wurden.

K. NAKAO and S. M. LEVENSON

Departments of Pathology and Surgery and Germfree Research Program, Albert Einstein College of Medicine, Yeshiva University, New York 61 (N.Y. 10461, USA), 16th December 1966.

[19] Y. AVI-DOR, Biochem. biophys. Acta *39*, 53 (1960).
[20] P. SIEKEVITZ and G. E. PALADE, J. biophys. biochem. Cytol. *4*, 309 (1958).
[21] B. F. TRUMP, P. J. GOLDBLATT and R. E. STOWELL, Lab. Invest. *11*, 986 (1962).
[22] This work was supported in part by Grant No. 5 POI AM05664-05 AMP and Grant No. 5-K6-GM-14,208-05 to the Albert Einstein College of Medicine by the National Institutes of Health.

Specificity of the Macrophage Reaction in vitro

BOYDEN[1] demonstrated that macrophages, from guinea-pigs with delayed hypersensitivity to sheep red cells, give mixed agglutination in vitro, forming a 'rosette' with these red cells. Sera of these animals contain a cytophilic antibody similar to the one shown in sera of immunized rabbits[1–5]. These sera confer in vitro, to macrophages of normal guinea-pigs, ability to absorb on their surface the soluble or cellular antigens used for immunization. Analogous results were reported by JONAS et al.[6].

We have investigated the species- and the group-specificity of this phenomenon. For this purpose groups of guinea-pigs were inoculated with human red cells (A, B, O), human sera (A, B, O) and sheep red cells respectively. The red cells and the sera mixed with complete Freund Adjuvant (Difco) were inoculated into the footpads. Skin tests, subsequently performed with urea extract of the appropriate red cells[1] or with human sera, showed a delayed reaction of hypersensitivity.

Macrophages were obtained from the peritoneal cavity, 2 weeks after inoculation, by injecting i.p. 15 ml of 199*M*[7] with 1% heparin; the abdomen was massaged for 3 min, the peritoneal fluid sucked up in sterile siliconized pipettes, transferred to siliconized tubes, washed 3 times and resuspended[8] in fresh 199*M*. About 70% of the cell population consisted of macrophages.

Macrophages from guinea-pigs inoculated with different red cells were brought into contact in vitro with the erythrocytes used for immunization or with heterologous ones. Macrophages from guinea-pigs inoculated with human sera (A, B, O) were divided into 2 groups: the first was incubated in vitro with these sera and, after washing, brought into contact with human red cells from different blood groups; the second was brought into contact, in vitro, with human red cells (A, B, O), without being previously exposed in vitro to any of these sera.

Examination using phase-contrast microscopy, showed the following: macrophages from guinea-pigs inoculated with red cells invariably gave a strong positive reaction in vitro with the red cells used for inoculation (Figure 1a). Macrophages from guinea-pigs inoculated with sheep red cells mostly gave strong in vitro agglutination with sheep red cells but some provoked adherence of a few rabbit and human red cells. Agglutination of rabbit and human red cells was slow (12–20 h), whereas that of sheep red cells was more rapid (2–3 h). Goat, horse, ox and swine red cells were not adsorbed.

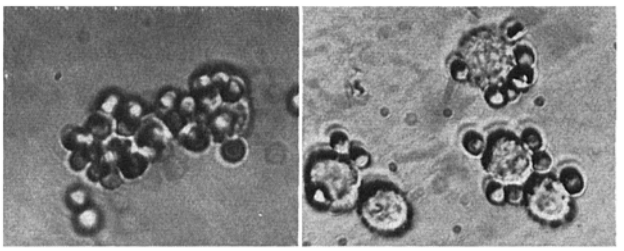


Fig. 1. Left: Adherence in vitro of sheep red cells, on macrophages of guinea-pig inoculated with sheep red cells. Right: Adherence of human red cells on macrophages of guinea-pig inoculated with pool of human sera, after previous in vitro sensitization of macrophages with human sera.

[1] S. V. BOYDEN, Immunology *7*, 474 (1964).
[2] S. V. BOYDEN, in *Cell bound antibodies* (Ed. B. AMOS and H. KOPROWSKI; Wistar Inst. Press. Philadelphia 1963), p. 7.
[3] S. V. BOYDEN and E. SORKIN, Immunology *3*, 272 (1960).
[4] S. V. BOYDEN and E. SORKIN, Immunology *4*, 244 (1961).
[5] E. SORKIN, in *The immunological competent cell*, a Ciba foundation symposium (Ed. G. E. W. WOLSTENHOLME and J. KNIGHT; Churchill Ed. London 1963), p. 38.
[6] W. E. JONAS, D. S. GURNER, D. S. NELSON and R. R. A. COOMBS, Int. Archs Allergy appl. Immun. *28*, 86 (1965).
[7] H. J. MORTON, J. F. MORGAN and R. C. PARKER, in *Methods of tissue culture*, 3rd edn (Ed. R. C. PARKER; Hoeber P. B. Inc. New York 1963), p. 74.
[8] J. SCHWARTZ, A. KLOPSTOCK, P. ZICKERT-DUVEDEVANI and S. HONIG, Int. Archs Allergy, appl. Immun. *26*, 333 (1965).