turbidity and by phase contrast microscopy). The thickness of the cell walls of *B. megaterium* when measured in ultra-thin sections was about 310 Å. After formamide extraction it dropped to 110 Å. At the poles of some membranes, remnants of the surface layer were found, this being evidence for the superficial localization of the removed layer (teichoic acid in the case of *B. megaterium*, Figure 2). Considering some literary data[2,3] and the lysozyme sensitivity of the fine membranes, it may be assumed that they represent the mucopeptide basal layer of *B. megaterium* and of *B. subtilis*, respectively. Detailed results, together with a conception of the ultrastructural arrangement of the Gram-positive cell wall, will be published elsewhere[7].

Gram-negative bacteria, 18-h-old cells of *Proteus vulgaris* P_2 (CCM 1799), were treated with formamide at 170°C for 20 min or longer. In no case were regular rod-shaped membranes observed. On the contrary, irregular, partly solubilized material with remnants of another substance was found (Figure 3). This unsuccessful attempt to isolate the mucopeptide layer could be explained by stronger covalent bonds between the mucopeptide and a substance of the overlying layer (peptides or lipopolysaccharides)[7–10].

Zusammenfassung. Die obere Schicht der Zellwände von *B. megaterium* M und *B. subtilis* wurde mittels Extraktion mit heissem Formamid beseitigt. Zurückbleibende stäbchenförmige, etwa 110 Å dicke Membranen entsprechen offenbar der rigiden Mucopeptid-Basalmembran der Bakterienzellwände.

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[7] M. V. NERMUT, in press.
[8] J. MANDELSTAM, Biochem. J. *84*, 294 (1962).
[9] H. H. MARTIN and H. FRANK, Z. Naturf. *17b*, 190 (1962).
[10] R. PLAPP and O. KANDLER, Archiv Mikrobiol. *50*, 171 (1965).

Metabolism of Phospholipids in Scorbutic Guinea-Pigs

The role played by *l*-ascorbic acid in the metabolism of phospholipids is as yet quite unexplored. There are a number of works concerned with the catalysing effect of vitamin C in phospholipid oxidation (ELLIOTT and LIBET[1], WILLIAMS[2], RADSMA and VAN GRONINGEN[3]); but there is only a very small amount of work on the metabolism of phospholipids in organisms subjected to C-avitaminosis.

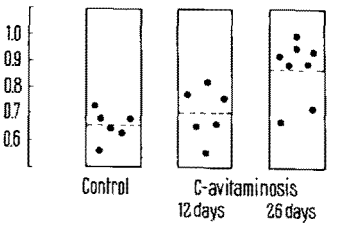
50 male guinea-pigs, weighing 200 g on the average, were divided into 4 groups and fed *ad libitum* on a slightly modified Lunde scorbutogenic diet. The control group obtained daily 5 mg *l*-ascorbic acid perorally. The experimental animals were killed on the 9th, 18th and 25th day respectively of the scorbutogenic regime, and subsequently the concentration of phospholipids was determined according to the method of STEWART and HENDRY[4] in their blood serum and liver. In further experiments, on a group of control animals, guinea-pigs fed for 12 and 26 days on scorbutogenic diet, $Na_2HP^{32}O_4$ was administered intraperitoneally in doses of 0.16 μC for 1 g of their weight; 24 h later the animals were killed, and phospholipids were isolated from their liver according to the method of DAVIDSON et al.[5]. In the fraction obtained, the phosphorus content was determined by means of phosphomolybdenate and also the radioactivity was determined. The results were expressed in the form of relative specific activity given by the relationship of the specific activity of phospholipids to the specific activity of acid-soluble phosphorus.

The changes which occurred in the concentration of phospholipids in the course of C-avitaminosis are shown in the Table. At the beginning a very significant increase can be seen in the blood serum, returning to normal not earlier than in the terminal phase of scurvy. In the liver, a provable decrease takes place until the 9th day of the avitaminosis; up to the 18th day the level increases, and continues to increase until the terminal phases of C-avitaminosis.

On studying the incorporation of P^{32} into liver phospholipids, it was found that this process undergoes no change during a 12-day administration of scorbutogenic diet; on the other hand, under conditions of an already developed C-avitaminosis (in a phase when the animals refuse food) a provable acceleration of the process ($P < 0.01$) takes place (Figure).

Concentration of lipid phosphorus (mg %) in blood serum and liver of guinea-pigs during the development of C-avitaminosis

		Blood serum	Liver
Control		2.8 ± 0.2	134.2 ± 2.8
C-avitaminosis	9	7.0 ± 0.2	109.2 ± 1.7
days	18	5.8 ± 0.4	148.5 ± 8.5
	25	3.5 ± 0.4	204.3 ± 4.6



Relative specific activity of liver phospholipids in normal and scorbutic guinea-pigs.

[1] K. A. ELLIOTT and B. LIBET, J. biol. Chem. *152*, 617 (1944).
[2] J. N. WILLIAMS, Proc. Soc. exp. Biol. Med. *77*, 315 (1951).
[3] W. RADSMA and H. E. M. VAN GRONINGEN, Acta physiol. pharmac. néerl. *8*, 15 (1959).
[4] C. P. STEWART and E. B. HENDRY, Biochem. J. *29*, 1683 (1935).
[5] J. N. DAVIDSON, S. C. FRAZER, and W. C. HUTCHINSON, Biochem. J. *49*, 311 (1951).

The changes occurring in phospholipid levels in the different tissues of scorbutic guinea-pigs are not uniform (KAWISHWAR et al.[6]); concentration of lipoid phosphorus increases in the blood serum (BANERJEE and BANDYOPADHYAY[7]). Our findings are in agreement with these results. In view of the central role played by the liver in phospholipid metabolism, a most interesting finding is the marked accumulation of phospholipids in the liver of scorbutic guinea-pigs. Simultaneously with the increase of phospholipid concentration, an increased incorporation of the phosphate P^{32} into the fraction of lipoid phosphorus also takes place. This signifies that, in conditions of fully developed C-avitaminosis, an increased hepatal synthesis of phospholipids occurs. However, the mechanism of this phenomenon remains undetected, and it is not excluded that the accumulation of liver phospholipids depends also on an increased deposition of phospholipids in the liver to the detriment of other organs.

Zusammenfassung. Akuter Vitamin C-Mangel verursacht bei Meerschweinchen vorübergehenden Anstieg des Phospholipidspiegels im Blutserum, erhöhte Inkorporation des P^{32}-Phosphates in die Leberphospholipide und eine markante Akkumulation von Leberphospholipiden.

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[6] W. K. KAWISHWAR, B. CHAKRAPANI, and S. BANERJEE, Indian J. med. Res. *51*, 488 (1963).

[7] S. BANERJEE and A. BANDYOPADHYAY, Proc. Soc. exp. Biol. Med. *112*, 372 (1963).

5-Methoxy- and 5-Hydroxy-Indolealkylamines in the Skin of *Bufo alvarius*[1]

Amongst the 40 *Bufo* species so far examined in this laboratory for their skin content of biogenic amines, the North American *Bufo alvarius* Girard occupies a unique position. The skin of this desert toad contains, in addition to the usual 5-hydroxyindolealkylamines and to several unknown indoles, enormous amounts of 5-methoxyindole derivatives represented mainly by 5-methoxy-N,N-dimethyltryptamine (*O*-methylbufotenine) and, subordinately, by 5-methoxy-N-methyltryptamine and 5-methoxyindolacetic acid (5-MIAA).

The dry skins of 12 specimens of *Bufo alvarius* were extracted twice with 70% acetone, always keeping distinct the glands and the remaining skin. A minor part of the 24 extracts was put aside for individual estimations, the bulk was pooled to constitute a glandular and a non-glandular skin extract, respectively. Amounts of these extracts corresponding to 20 g of non-glandular skin, or 5 g of glands, were evaporated to dryness and the residues taken up in 98% ethanol, which was then passed through an alkaline alumina column. Elution was carried out, as usual, with descending concentrations of ethanol, and the different eluates submitted to paper chromatography, thin layer chromatography and bioassay[2]. It was found that cutaneous glands contained 0.8 to 5 mg bufotenine and as much as 60 to 160 mg *O*-methylbufotenine per g dry tissue. For non-glandular skin, values of known compounds were as follows: 0.33–2.15 mg bufotenine, 1.0–3.5 mg *O*-methylbufotenine, 0.020–0.023 mg 5-methoxy-N-methyltryptamine, 0.04 mg 5-MIAA, 0.004–0.006 mg 5-HT, 0.03–0.04 mg N-methyl-5-HT, and 0.011–0.012 mg/g 5-HIAA. *O*-methylbufotenine could easily be obtained in crystalline form, as picrate, from the 98% ethanol eluate of the alumina column loaded with the glandular extract. This picrate was indistinguishable from the corresponding synthetic compound. The base prepared from the natural picrate and the synthetic *O*-methylbufotenine base, in their turn, showed the same chromatographic behaviour, the same ultraviolet and infrared spectra and the same peak in gas chromatography (HOLMSTEDT et al.[3]), and on treatment with hydrogen peroxide gave the same *N*-oxide.

So far, 5-methoxyindoles have been found only in some South American vegetables[3–6] and, in animals, only in the pineal gland of mammals and birds[7,8]. According to AXELROD and WEISSBACH[9], 5-hydroxyindole-*O*-methyltransferase, the enzyme responsible for *O*-methylation of 5-hydroxyindoles, is strictly localized in the pineal body. Present results show that amphibian skin also may possess 5-hydroxyindole-*O*-methyltransferase activity.

Details will be published elsewhere.

Riassunto. Gli estratti di pelle di *Bufo alvarius*, rospo delle regioni desertiche del Nord America, contengono, oltre alle consuete 5-idrossiindolalchilamine (5-HT, N-metil-5-HT, bufotenina) enormi quantitativi di derivati 5-metossiindolici, rappresentati soprattutto da *O*-metilbufotenina. Questa può giungere a costituire fino il 16% del peso delle ghiandole cutanee secche. Si insiste sulla probabile presenza di 5-idrossiindolo-*O*-metiltransferasi nella pelle di *Bufo alvarius*.

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[1] Supported by a grant from the Consiglio Nazionale delle Ricerche, Roma.

[2] Standard 5-methoxyindoles used for comparison were in part obtained from the Farmitalia Research Laboratories, Milan; in part synthesized by one of us (V.). A sample of natural *O*-methylbufotenine was kindly supplied by Dr. TSCHESCHE, Bonn.

[3] B. HOLMSTEDT, V. J. A. VANDENHEUVEL, W. L. GARDINER, and E. C. HORNING, Analyt. Biochem. *8*, 151 (1964).

[4] S. WILKINSON, J. chem. Soc. *2*, 2079 (1958).

[5] I. PACHTER, D. E. ZACHARIUS, and O. RIBEIRO, J. org. Chem. *24*, 1285 (1959).

[6] G. LEGLER and R. TSCHESCHE, Naturwissenschaften *50*, 94 (1963).

[7] A. B. LERNER and Y. TAKAHASHI, J. biol. Chem. *235*, 1992 (1960).

[8] J. AXELROD, R. J. WURTMAN, and CH. M. WINGET, Nature *201*, 1134 (1964).

[9] J. AXELROD and H. WEISSBACH, Science *131*, 1312 (1960); J. biol. Chem. *236*, 211 (1961).