Organism	Ref.	Methanol/alcohol dehydrogenase	Pteridine content μM/g Cell (dry weight)	μM/g Protein
Pseudomonas M27	1	+	0.29	0.24
Pseudomonas methanica	6	+	0.14	
Methylococcus capsulatus (Bath)	7	+	0.10	0.33
Methylococcus capsulatus (Texas)	8	+	0.76	0.37
Hyphomicrobium sp.	9	+	0.46	
Methylosinus trichosporium (strain PG)	7	+	0.25	0.075
Methylosinus sporium (strain 5)	7	+	0.37	
Methylobacter sp.	7	+	0.30	
Pseudomonads				
Yellow organism	10	+	0.07	
White organism	10	+	0.10	
Pink organism (grown on methanol)	10	+	0.24	
Pink organism (grown on succinate)		+	0.15	
Pink yeast	10	−	0.09	
Arthrobacter sp.	10	−	0.27	0.108
Escherischia coli	3	not determined	0.5	
Zyotobacter vinelandii	3	not determined	0.020	
Anacystis nidulans	3	not determined	6.8	
Chromatium D	3	not determined	0.20	

substantially higher than is found in these organisms. A few values were calculated in terms of the protein content of the cells but, although this resolved the apparent contradiction between the two strains of *Methylococcus capsulatus* (the 'Bath' strain must contain a large amount of non-proteinaceous material), it did not show any other significant trends.

The pteridines in the *Pseudomonas sp.* (pink organism) have been identified using methods described previously. The 4 compounds isolated from this organism are neopterin cyclic phosphate[4], 2-amino-4-hydroxy-6-carboxypteridine, neopterin, and 2-amino-4-hydroxy-6-methylpteridine. They were isolated in the ratio 3.5:2:1:4.7, respectively. These have also been isolated from *M. capsulatus*[4], and, apart from the cyclic phosphate, from *Methylosinus sporium* strain 5 and *Methylosinus trichosporium* strain PG[5]. There thus appears to be considerable similarity in the compounds occuring in these bacteria[11].

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[12] Deceased, May, 1971.

Zusammenfassung. Der Pteridin-Gehalt einiger Methan-oder Methanol-oxydierender Bakterien scheint nicht mit der Anwesenheit einer spezifischen Methanoldehydrogenase zusammenzuhängen. Pteridine einer *Pseudomonas* Sp. (pink organism) wurden identifiziert.

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An Urinary Metabolite of Bromazepam

Bromazepam[1], 7-bromo-1, 3-dihydro-5(2-pyridyl)-2H-1, 4-benzodiazepin-2-one, is a member of the 1, 4-benzodiazepines such as chlordiazepoxide and diazepam. It is now under clinical evaluation as a psychotropic drug in the control of ambulatory schizophrenics. The present investigation was undertaken in order to isolate and to characterize urinary metabolites of bromazepam administered to dogs as well as to rabbits. The drug was administered orally in a single dose of 200 mg/kg to rabbits and 50 mg/kg to dogs. Urine was collected during the following 48 h and hydrolysis of glucuronides was carried out by the usual method employing β-glucuronidase. The hydroly-

[1] Bromazepam (Ro 5-3350) is synthesized by R. IAN FRYER, R. A. SCHMIDT and L. H. STERNBACH, Department of Chemical Research, Hoffmann-La Roche, Inc., Nutley, N. J., USA (J. Pharm. Sci., *53*, 264, 1964).

Rf values and colour reactions of bromazepam and its metabolites in thin-layer chromatography

Substance	Solvent system I	II	III	Color reaction Dragendorff	Bratton-Marshall	Phenol reagent
Bromazepam	0.05	0.05	0.53	orange	purple[a]	—
2-Amino-5-bromobenzoylpyridine	0.74	0.60	0.85	orange	purple	—
Unknown metabolite	0.58	0.34	0.84	orange	grayish purple	blue[b] bluish gray[c]

Adsorbent: Silica Gel G (Merck). Solvent system: I, Chloroform-ether (3:1); II, Chloroform-acetone (9:1); III, Ethyl acetate. [a] Spray with the Bratton-Marshall reagent after hydrolysis by 2*N* H_2SO_4. [b] Potassium ferricyanide-ferric chloride reagent. [c] Folin-Ciocalteu reagent.

zate was adjusted to pH 8.0 and extracted with ethyl acetate. The extract was first dried over anhyd. sodium sulfate and then evaporated in vacuo. The residue was dissolved in a small amount of ethyl acetate and was subjected to thin-layer chromatography. The Table shows the Rf values and colour reactions of bromazepam and its metabolites. The residual part was chromatographed on a column of Silica Gel by successive elution with chloroform-ether (3:1). The eluate was then submitted to rechromatography on a Silica Gel column. Elution with the same solvent system and recrystallization of the eluate from chloroform yielded ca. 20 mg of an unknown metabolite. The compound was obtained as orange prisms of m.p. 204–206° (decomp.). Thin-layer chromatography with the solvent system mentioned in the Table revealed a discrete spot without tailing. Anal. calcd. for $C_{12}H_9O_2N_2Br$: C, 49.17; H, 3.09; N, 9.56. Found: C, 49.27; H, 2.95; N, 9.81. UV $\lambda_{max}^{50\%\,\text{aq. EtOH}}$ nm ($\log \varepsilon$): 238 (4.24), 280 shoulder (3.89), 410 (3.61). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 3300 (NH_2), 1630 (C=O). NMR (in DMSO-d_6) δ: 7.02 (1H, doublet, J = 2Hz, aromatic C_4–H), 7.17 (1H, doublet, J = 2Hz, aromatic C_6–H), 8.15 (4H, multiplet, pyridyl C–H, this pyridyl aromatic protone signal was not changed from the protone signal of 2-amino-5-bromobenzoylpyridine). Mass Spectrum *m/e*: 292 (M^+). From the spectral and elemental analysis data, the structure of the metabolite under investigation was assumed to be a 3-hydroxylated derivative of 2-amino-5-bromo benzoylpyridine. For the purpose of identifying the position of the hydroxy group, the synthesis of the authentic substance is now under way. It appears that the majority of the metabolite was excreted as the glucuronide conjugated form in the urine, it may be concluded that bromazepam itself, or after opening of the benzodiazepine ring, undergoes a process of hydroxylation. Details will be published in the near future.

Zusammenfassung. Es wird über Versuche zur Isolierung von Metaboliten im Harn des Kaninchens und des Hundes nach einmaliger oraler Gabe von Bromazepam in hohen Dosen berichtet. Die Ergebnisse der Elementaranalyse und des UV-, IR-, NMR- und Massenspektren deuten darauf, dass es sich um das 3-Hydroxy-Derivat des 2-Amino-5-brombenzolypyridin handelt.

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The Penetration of the Membrane of Mitochondria, obtained from Livers of Adult or Old Rats, by Different Anions

The anion permeability of mitochondrial membranes has been widely investigated. It was shown, in experiments performed by measuring mitochondrial swelling, that chloride and fumarate penetrated mitochondria poorly, but citrate, malate, phosphate, and succinate penetrated well with the aid of specific translocases. The permeability of citrate, malate and succinate also required a small amount of phosphate, in addition to which the citrate penetration required a small amount of malate[1,2].

SPENCER and LÖWENSTEIN[3] stressed the importance of citrate ion translocation from the mitochondrion into the cytoplasm to insure the normal fatty acid synthesis. ABRAHAM et al.[4] and others[5] found a decreased fatty acid synthesis in the liver cells of adult rats which developed hyperlipaemia. In the case of old animals, however, an increase instead of this decrease was observed[6]. For these reasons it seemed worth investigating the swelling of liver mitochondria obtained from normal and hyperlipaemic rats of different ages in the presence of different anions.

Methods. Experiments were made on 44 adult (4–6-month-old) and 28 old (24–26-month-old) Wistar inbred male rats kept on 15 g semisynthetic diet daily. One half of all age-groups were neurotized according to a fixed scheduled program[7]. After the completion of the pro-

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