were seen. It was expected that after alkali treatment pores, if present, would be more evident.

SHATKIN and TATUM[7] suggested that septum formation in *Neurospora* occurs by a process of cell wall invagination and fusion. Whether this is the case in *G. lactis* is unknown. However, it is clear that the discs corresponding to septa are readily released from the walls of the pseudomycelium. In the electron microscope, a great number of these discs, more or less free of cell walls, can be seen. The liberation of these septa is probably due to partial chemical digestion of the junction between septum and wall.

These septa correspond to fraction 1 of KESSLER and NICKERSON[2], that is the cell wall after digestion with alkali. Theoretically these should contain only the glucan-protein component, but we have also found mannose in this fraction.

Resumen. Las pareces celulares aisladas de *G. lactis*, después de tratadas con éter y KOH 1 *N*, muestran al examinarles en el electrónico, los tabiques transversales, circulares, de estructura fibrilar no orientada y sin poros, separados del resto de la pared. Su composición es glucano-proteina y manosa.

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Preliminary Studies of the Metabolism of 2-Guanidinomethyl-1:4-benzodioxan Sulphate (Guanoxan)

Guanoxan is a powerful antihypertensive agent in man, and in dogs produces a considerable adrenergic neurone blockade, antagonizing the effects of epinephrine and norepinephrine at α-receptor sites, depleting the norepinephrine content of the heart, spleen and hypothalamus and adrenal glands[1–5].

The metabolism of 2-alkylaminomethyl-1:4-benzodioxan has been studied in man and in animals in detail by MCMAHON et al.[6–8]. Oxidative degradation of the alkylaminomethyl chain and hydroxylation of the aromatic nucleus were the main metabolic pathways found. Although guanidines which exist normally in biological systems undergo many transformations, there is very little evidence, if any, to show that other non-physiological guanidines are metabolized to a great extent. Thus, when taurocyamine[9] is given to dogs, 80% of it is excreted unchanged; sulphaguanidine[10] is acetylated in the amino group of the aromatic ring and streptomycin and dihydrostreptomycin[11] are excreted largely unchanged.

Preliminary recovery experiments with non-radioactive guanoxan showed that it could be extracted with *n*-butanol from salt-saturated urine and assayed colorimetrically by means of the Voges-Proskauer diacetyl reagent for guanidines[12]. When a single dose of guanoxan (88 mg) was administered orally to a beagle dog, the total excretion of the unchanged drug during the 4 days consecutive to dosage was found by the colorimetric assay to be 21–22%.

Guanoxan-(guanidino-^{14}C) was prepared from 2-aminomethyl-1:4-benzodioxan by reaction with S-methyl thiouronium sulphate-(uronium-^{14}C). A Packard Tricarb

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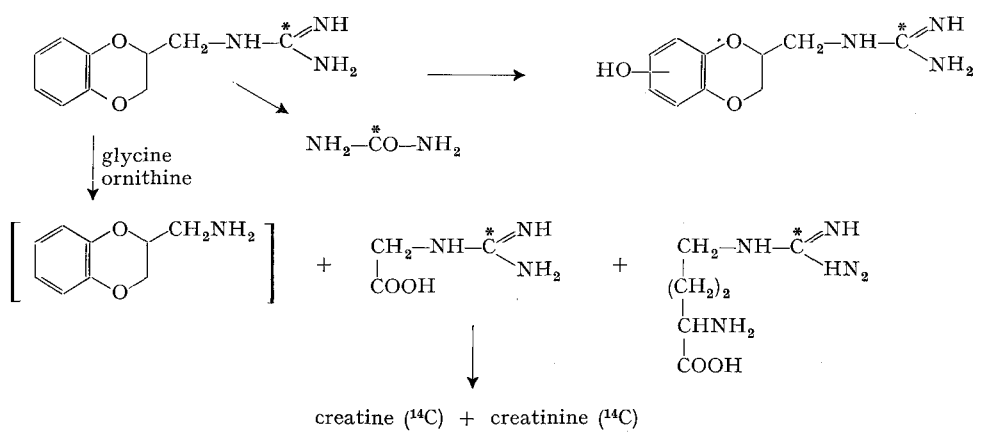

scintillation counter was used for determining the radioactivity.

24 daily doses (20 mg/kg) of unlabelled guanoxan were given orally to a male beagle dog (11.5 kg). These were followed by a similar dose of radioactive guanoxan (3.24 μc). The urine was then collected over a period of 4 days, concentrated in vacuo and extracted with methanol. Radioactivity measurements at this point indicated that the recovery of ^{14}C-labelled metabolites was in the order of 52–53% of the administered radioactive dose.

The fractionation of the ^{14}C-labelled metabolites was carried out by two different methods simultaneously: (a) absorption on Amberlite IRC 50 resin, buffered to pH 6.5 with disodium hydrogen phosphate, then elution of the metabolites with water, 0.5 *N* HCl and 1.2 *N* HCl in methanol successively; (b) preparative thick paper descending chromatography using *n*-propanol-aqueous molar ammonium carbonate-concentrated ammonia (0.88) (70:30:2) as the developing solvent.

The identification and characterization of the metabolites were achieved by direct comparison of the Rf values with those of standards using chromatographic, electrophoretic and combined bidimensional techniques, and also by the preparation of solid derivatives (reineckates, picrates) and by comparison of these with authentic derivatives.

Fractionation by method (a) gave the following labelled metabolites: arginine, 4.3%; creatine-guanidinoacetic acid, 12%; creatinine-urea, 19%; hydroxylated guanoxan, 4%; guanoxan 13.6%. Fractionation according to (b) afforded the same metabolites, but better separation was achieved between creatinine and urea: arginine, 5%; creatine-guanidinoacetic acid, 9.5%; creatinine, 15.5%; urea, 3.6%; hydroxylated guanoxan, 4.5%; and guanoxan, 15%.

These results suggest that the guanidino group of guanoxan underwent transamidination in the presence of ornithine and glycine, to give arginine and guanidino acetic acid respectively, the latter giving rise to creatine and creatinine. Hydrolysis of the guanidino group to urea does not appear to be very extensive judging by the small amount of labelled urea found (3–4%). It is assumed that desimidation does not take place because the metabolic product, *N*-[2(1,4-benzodioxanyl)methyl]urea, was not detected even in traces. The position of the hydroxylation in the aromatic ring was not established fully, but the chromatographic behaviour of a phenolic metabolite was identical to that of synthetic 2-guanidinomethyl-7-hydroxy-1,4-benzodioxan sulphate, m.p. 263–264.5°.

The latter was prepared from 2-chloromethyl-7-hydroxy-1,4-benzodioxan[13] by conversion to the azide (b.p. 158–164°/0.6 mm) followed by hydrogenation to the 2-aminomethyl-7-hydroxy-1:4-benzodioxan (hydrogen maleate, m.p. 184–185°) and guanylation with 1-amidino-3,5-dimethylpyrazole sulphate.

The metabolic fate of the benzodioxan ring system will be discussed in the future.

Resumen. El sulfato de 2-guanidinometil-1:4-benzodioxano (guanidino ^{14}C) se sintetizó a partir del 2-aminometil-1:4-benzodioxano y el sulfato de S-metiltiouronio (uronio ^{14}C). Un 52–53% de la dosis radioactiva administrada a los perros se recuperó en la orina en la forma de los siguientes compuestos radioactivos: arginina, creatina, acido guanidino acético, creatinina, urea, 2-guanidinometil-1:4-benzodioxano y un derivado hidroxilado (fenólico) del anterior. Se sintetizó tambien el sulfato de 2-guanidinometil-7-hidroxi-1:4-benzodioxano.

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Phosphorylation of Thiamine in the Intestinal Wall During Absorption in vivo

Several experiments in vitro[1–3] have shown that intestinal tissue is able to phosphorylate thiamine. The possibility that thiamine is phosphorylated during its intestinal absorption has been suggested by some authors[4,5] and denied by others[6]. However, a clear experimental evidence of the relationship between thiamine phosphorylation and its intestinal absorption has never been produced, although Machida[7] found some thiamine phosphates in the wall of isolated intestinal tracts of the rat after incubation with thiamine.

Recently Ventura and Rindi[8] were able to show an uphill transport of thiamine by the everted intestinal sacs of the rat in vitro, and put forward the hypothesis that the underlying mechanism of transport could be thiamine phosphorylation.

Here we will refer to some results we obtained in an in vivo study of thiamine phosphorylation during the intestinal absorption of equivalent amounts of thiamine hydrochloride and thiamine-propyl-disulphide (TPS), a well-known thiamine derivative rapidly absorbed and completely transformed into thiamine by the intestinal mucosa[9–11].

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