Identification of Creatine by Partition Chromatography

IN a study of creatinine metabolism in the rat, to be published elsewhere, creatinine has been identified qualitatively by means of the technique of partition chromatography on paper strips developed by Consden, Gordon and Martin[1]. The apparatus described by Dent[2] has been used, with the further refinement of accurate temperature control obtained by immersing the outer glass container in a water thermostat at 15 ± 0·05° C. Solutions containing *c.* 20–30 γ of creatinine were pipetted on to the paper strips, and after treatment with an appropriate solvent, for example, collidine for 24 hours, the creatinine bands were located by spraying with alkaline sodium picrate.

Some rat urines examined in this manner showed, in addition to a creatinine band, a further Jaffe positive band corresponding to a substance of lower R_f value than creatinine. The second band has since been shown to arise from the creatine, present in the urine, which had undergone movement in the solvent and which had been dehydrated to creatinine during the drying of the paper strips. By means of acid treatment of the strips it is possible to convert creatine more completely to its anhydride *in situ*, and in this way to determine R_f values for creatine.

Further, this procedure affords a method for determining creatine qualitatively in the presence of creatinine, the method adopted being as follows: the test material is pipetted on to the strip and is treated with the appropriate solvent. The strip is dried at 100° C., sprayed with 0·5 *N* sulphuric acid and again heated at 100° C. for 1 hour. Creatine is located on the strip as creatinine by spraying with alkaline sodium picrate. R_f values determined at 15° C. for creatine and creatinine (solvent collidine) both in pure solutions and in rat urine are given in the accompanying table.

	Pure solution	Rat urine
Creatine	0·19	0·15
Creatinine	0·47	0·57

Each figure represents an average of at least six values, the maximum variation obtained being 9 per cent. The R_f values for creatine are sufficiently far removed from those of creatinine to enable clear-cut separation of these substances, and it is therefore possible to identify creatine qualitatively in the presence of considerable amounts of its anhydride.

Further solvents giving suitable R_f values for these two substances are being tried. Creatine and creatinine in phenol move as fast as the solvent. Creatinine shows no movement in amyl alcohol, but has a low R_f value in *n*-butyl alcohol (creatinine in pure solution: 0·20; creatinine in rat urine: 0·25).

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[1] Consden, R., Gordon, A. H., and Martin, A. J. P., *Biochem. J.*, **38**, 224 (1944).

[2] Dent, C. E., *Lancet*, ii, 637 (1946).

Enzymatic Hydrolysis of Cellulose by *Coniophora cerebella*

DURING the course of the investigation of the enzymatic activities of wood-destroying moulds[1], an attempt was made to depict the phase sequence of the degradation of the cellulosic fraction of wood; cellulose itself, in the forms of filter paper and surgical cotton, was utilized. *Coniophora cerebella* was found to degrade both forms of cellulose to oxalic acid. Inasmuch as traces of reducing material were found to be formed, and oxalic acid was also obtained by the action of the fungus on glucose, it appeared that an introductory hydrolysis was a necessary prerequisite for the formation of oxalic acid.

A hydrolytic action being involved, it was found useful to examine the residual cellulosic material to determine whether there was an increase or decrease in reducing power. If one were to assume that a hydrolysis similar to that by acids took place, then there would be an increase in reducing power due to the formation of new reducing groups. On the other hand a split of semi-acetal bonds, the existence of which was first surmised by Tollens, might take place, with the result that the residual material would show a lower reducing value.

Some experiments by Hiller and Pacsu[2] indicated that an *initial* rapid acid hydrolysis of cellulose causes a split of semi-acetal bonds, permitting a *decrease* in reducing power. Consequently, speculation arose whether an enzymatic degradation of cellulose would follow such a pattern, or whether the mould effects a split of 1,4-glycosidic bonds to give rise to new reducing groups.

Surgical cotton which was degraded by *Coniophora cerebella* over a period of sixty days was examined at certain intervals, employing the analytical technique of Hiller and Pacsu, which requires the use of dilute acidic permanganate solution for the determination of reducing power. An inspection of the values recorded in the accompanying table indicates an *increase* in reducing power of the residual cellulose as the oxalic acid content increases:

DISSIMILATION OF SURGICAL COTTON BY *Coniophora cerebella*

Incubation period Days	Oxalic acid (mgm.)	Milli-equivalents oxygen used up
0	0	0·0500
13	2·35	0·1201
30	6·30	0·2612
60	6·40	0·2668

Values expressed per 1 gm. samples of surgical cotton.

It would appear that the *enzymatic* degradation of cellulose effects a split of 1,4-glycosidic bonds rather than, or concomitant with, a split of semi-acetal linkages.

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[1] Nord and Vitucci, *Nature*, **160**, 224 (1947).

[2] Hiller and Pacsu, *Text. Research J.*, **16**, 490 (1946).

Synthesis of the Antibiotic, Penicillic Acid

PENICILLIC acid, first isolated by Alsberg and Black[1] from *Penicillium puberulum* Bainier and later by Birkinshaw, Oxford and Raistrick[2] from *Penicillium cyclopium* Westling, was shown to possess the structure (IV) by the latter authors. Later it was observed that the compound was a powerful antibacterial agent, and Oxford[3] has determined that it is much more active than penicillin against Gram-negative organisms.

Penicillic acid has now been synthesized from the lactone (I)[4] as follows:

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