Effect of Hormones on Renal Gluconeogenesis

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It was demonstrated previously that glucose production in the kidney cortex was increased in diabetic, growth-hormone-treated and cortisone-treated rats (Joseph & Subrahmanyam, 1968). Pyruvate carboxylase (EC 6.4.1.1) activity, which was 3.20 ± 0.31 units in normal rats, was increased by 96% in the diabetic condition and by 67% by cortisone treatment. Growth-hormone treatment had no effect on the activity of this enzyme. Phosphoenolpyruvate carboxylase (EC 4.1.1.32) activity was increased by 49%, 58% and 69% in diabetic, cortisone-treated and growth-hormone-treated rats respectively. These findings, together with the previous finding of an elevated fructose 1,6-diphosphatase activity in all these conditions, explain the elevated glucose formation in the kidney cortex from gluconeogenic precursors.

The glycogen content of kidney cortex ($1.31 \pm 0.15 \mu\text{mol}$ of glucose equivalents/g wet wt.) was increased about fourfold in the diabetic condition and twofold by growth-hormone treatment. It was, however, unaltered by cortisone treatment. The activity of glycogen synthetase (EC 2.4.1.11) was not changed in any of these conditions. Phosphorylase (EC 2.4.1.1) activity was depressed by 53% and 41% respectively in the diabetic condition and by growth-hormone treatment. Cortisone treatment had no effect on this activity.

Thus the gluconeogenesis (glucose and glycogen production) in the kidney cortex of hormone-treated rats can be explained on the basis of enzyme activities.

Joseph, P. K. & Subrahmanyam, K. (1968) *Arch. Biochem Biophys.* 127, 288

Effect of L-Glutamate on Glucose Metabolism in Rat Cerebral Cortex

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Cerebral cortical slices of normal rats that had been injected with glutamate, glutamine and γ -aminobutyrate before they were killed were incubated with $[\text{U-}^{14}\text{C}]$ glucose. It was found that, whereas glutamate depressed the incorporation of the label from 9500 ± 608 to 5461 ± 350 c.p.m./g wet wt. of cerebral-cortex slice into glycogen and from

180760 ± 923 to 139116 ± 467 c.p.m./g wet wt. into CO_2 , there was no significant change as a result of injecting glutamine or γ -aminobutyrate.

One slice of cerebral cortex of normal rats was incubated with $[\text{U-}^{14}\text{C}]$ glucose and the slice from the other hemisphere with $[\text{U-}^{14}\text{C}]$ glucose + glutamate. The incorporation into glycogen was decreased from 9500 ± 608 to 4422 ± 503 c.p.m./g wet wt. and that into CO_2 from 180760 ± 923 to 84612 ± 1766 . Thus glutamate has a marked effect on the utilization of glucose in the cerebral cortex both *in vivo* and *in vitro*. Addition of insulin to the slices during incubation was not found to annul the depressing activity of glutamate on glucose metabolism.

In diabetic rats the incorporation into glycogen and CO_2 , which is already depressed (compared with normal slices), is markedly decreased further to about 20% for glycogen and 60% for CO_2 .

Studies on the permeability of radioactive glucose into the slices indicated that glutamate does not have any effect on this process. It is concluded that glutamate has a pronounced effect on the metabolism of glucose in the cerebral cortex of rats; this is being studied further.

Ligand-Induced Conformational Changes in Amylose, Studied by Spectrophotometric and Enzymic Methods

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Amylose is known to form helical complexes with butan-1-ol, cyclohexanol, dimethyl sulphoxide, iodine etc. The ligand-induced conformational changes in the polymer chain in an aqueous environment are detected by studying the influence of these complexing agents on the formation of the blue amylose-iodine complex (λ_{max} , 640nm) spectrophotometrically, and by the action of α -amylase (EC 3.2.1.1) and β -amylase (EC 3.2.1.2) on these as modified substrates. These complexing agents do not react with iodine in aqueous solutions at pH 4.8. The action of these enzymes on amylopectin is unaffected in the presence of complexing agents, as judged from the action pattern, optimum pH and K_m of these enzymes.

The influence of concentration of these complexing agents, time and temperature on the formation of the amylose-iodine complex is critically investigated. It is observed that there is a gradual increase in the absorption (λ_{max} , 640nm) of the blue amylose-iodine complex with increase in concentration of the complexing agent, reaching a maximum, followed by a decrease in the absorption, which in some cases is accompanied by a shift in λ_{max} , towards the blue region of the spectrum (640 to 600nm).

The amylose complexes are not degraded by α -amylase and β -amylase. The action of α -amylase and of β -amylase on amylopectin is inhibited in the presence of amylose complexes; the inhibition is of an uncompetitive type.

The increase in the absorption of the amylose-iodine complex in the presence of complexing agents, followed by a fall in the absorption at a higher concentration, accompanied by a blue shift in $\lambda_{\max}$, and the resistance to the action of α -amylase and β -amylase acquired by the amylose on complexing with these ligands appear to be due to the ligand-induced conformational changes.

Isolation and Characterization of High-Molecular-Weight Amylose by Dispersion of Starch Granules with Urea

By N. B. PATIL and N. R. KALE (*Department of Chemistry, Division of Biochemistry, University of Poona, Poona-7, India*)

Amylose, the linear component of starch, is susceptible to degradation in the presence of O_2 during dispersion of starch granules by heating, and hence dispersion of starch granules under anaerobic conditions or in the presence of dimethyl sulphoxide is recommended to avoid the degradation. Wet potato-starch granules were quantitatively dispersed in buffered urea (8–10M), pH 6.2, by prolonged contact at room temperature (26–28°C). Microscopic observations have shown that the starch granules in contact with urea are considerably swollen, accompanied by the loss of birefringence and followed by a progressive liberation of the starch components, leading to complete dissolution. Amylose is isolated by fractional precipitation with ethanol, followed by purification by complexing with butan-1-ol. Trace quantities of amylose in the amylopectin fraction are removed by adsorption on defatted cellulose. The purified components are recovered by addition of ethanol, and washed free of urea with aqueous ethanol. The amylose isolated by this procedure has the following properties: intrinsic viscosity, $[\eta]$, 3.56; iodine-binding capacity, 19.8, determined by a potentiometric method; blue value, 1.42, determined by a spectrophotometric method; β -amylolysis limit, 96–98%, indicating the high purity and high molecular weight of the linear polymer.

Factors Affecting Aflatoxin Production on a Synthetic Medium

By T. V. REDDY, L. VISWANATHAN and T. A. VENKITASUBRAMANIAN (*Department of Biochemistry, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi-7, India*)

A completely synthetic medium containing sucrose, asparagine and salts (SL medium) on which stationary cultures of *Aspergillus parasiticus* A.T.C.C. 15517 give high aflatoxin yields has already been reported from this laboratory. A detailed study was carried out on the effects of the concentrations of different components of SL medium and of other carbon and nitrogen sources on aflatoxin production. For optimal aflatoxin production, the ranges of sucrose, asparagine and ammonium sulphate concentrations were found to be 80–100, 10–15 and 2–5g/l respectively. Glucose, maltose and sucrose supported good aflatoxin production, whereas arabinose, fructose and starch were less effective. Of a number of the carbon sources studied, only glycerol and ethanol gave high yields.

The omission of Fe^{3+} , $Mo_7O_{24}^{6-}$ or H_3BO_3 from the medium had no marked effect on aflatoxin production. $ZnSO_4$ up to 25 mg/l stimulated aflatoxin formation, but considerable amounts of aflatoxins were formed even in the absence of Zn^{2+} . The omission of Mn^{2+} increased aflatoxin yields. Unlike many of the media recommended for aflatoxin production, SL medium gave equally good yields on both stationary and shake cultures.

Effect of Ionic Media on the Intestinal Transport of Amino Acids in Rats *in vitro*

By AKHTAR MAHMOOD and D. P. SHARMA (*Department of Biochemistry, Postgraduate Institute of Medical Education and Research, Chandigarh, India*)

Intestinal transport of methionine and glycine have been studied in rats by using the everted-sac technique. Both of the amino acids are actively transported by the intestinal epithelium and show an absolute requirement for Na^+ for their transport. Replacement of Na^+ by other ions of group I, namely Li^+ , Rb^+ and Cs^+ , in the incubation medium results in complete inhibition of the transport process, suggesting that Li^+ , Rb^+ and Cs^+ cannot substitute for Na^+ in the incubation medium. The results will be discussed in light of the recent concept of a polyfunctional membrane carrier, having different interacting sites for each of Na^+ , amino acids and sugars, located at the mucosal border of the small intestine.