Effect of carbohydrate source on cellulase activity of *C. aureum*

Medium[a]	Cellulase activity	
	Depth of clearing (mm)	N/200 $Na_2S_2O_3$ solution (ml)
Medium + glucose	—	—
Medium + glucose + cellulose	—	—
Medium + cellulose	5.92	14.39
Medium control	—	—

[a] Modified Czapek-Dox; -absence of activity.

tube (150 × 14 mm). For isolation and estimation of cellulase, *C. aureum* was grown in 75 ml of broth in 250 ml Erlenmeyer flasks.

Cellulose pulp[10] and filter paper (Whatman No. 1) were used as the cellulose source for stab and broth respectively. 0.7 mm diameter of inoculum disc of *C. aureum* grown on modified Czapek-Dox medium having cellulose pulp[10] as the only carbon source, and 0.5 ml of ascospores suspension (12.75×10^6), were used for stab and culture broth respectively. Incubation was done at 30 °C for 10 days. 5 replicates were considered for each observation.

It is evident from the Table that induction of cellulolytic enzyme was noticed only in cellulose supplemented medium. But in no case did glucose, and cellulose in combination with glucose, induce cellulase activity. This observation confirms that cellulase of *C. aureum* is not a constitutive enzyme but an adaptive one, since it is elaborated only in response to a specific substrate i.e., cellulose, as was observed in other organisms[1,2].

Zusammenfassung. Das cellulolytische Enzymsystem von *Chaetomium aureum* (Chivers) ist adaptiv. Seine Aktivität wird durch Cellulose, nicht aber durch Glukose oder Glukose plus Cellulose induziert.

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Effect of Allicin on Certain Enzymes of Liver After a Short Term Feeding to Normal Rats

In a recent paper[1], the authors have reported the lipid lowering effect of allicin on long-term feeding to normal rats. The authors have found also that allicin significantly lowered the blood sugar of alloxan diabetic rabbits, and that its effect was similar to that of tolbutamide[2]. The present paper deals with the effect of allicin on certain enzymes of liver after a short-term feeding of the drug to normal rats.

Material and methods. Four-month-old, young male wistar rats of average weight (125 g) were divided into 2 groups of 6 animals each. They were fed on normal laboratory diet as reported earlier[1]. Allicin was prepared from fresh garlic cloves, according to the method of CAVALLITO and BAILEY[3] for the present study. The initial fasting blood sugar level of all the rats was determined by the method of ASATOOR and KING[4]. Blood was collected from the tail. One group was kept as control and to the other group freshly prepared allicin (Dose 100 mg/kg/day) was orally administered as a solution in 2 ml distilled water. After 15 days treatment, the fasting (18 h) blood sugar was again determined as before in both the groups. Then they were sacrified by decapitation. Certain enzymes of the liver viz. hexokinase, α-glucan phosphorylase, glucose-6-phosphatase and lipase were determined by standard methods. Liver tissue was used for the enzyme preparation as reported from this laboratory[5].

Hexokinase (E.C. 2.7. 1.1. ATP. D-hexose-6-phosphotransferase) activity was determined by the measurement of disapperance of glucose in the presence of ATP[6]. The chilled liver tissue was homogenized at 0 °C with 3 volume of buffer of the following composition. *Tris*-0.1 *M*, histidine 0.1 *M*, EDTA-0.01 *M* and $MgCl_2$ 0.01 *M* (pH 7.0). The homogenate was centrifuged at 3000 × *g* at 0 °C for 5 min and the supernatant was used as the enzyme preparation. Enzyme activity was assayed by the method described by CRANE and SOLS[7]. One unit of enzyme activity is that amount which catalyse the phosphorylation of 1 μmol of glucose in 15 min at 30 °C under otherwise optimal conditions.

α-Glucan phosphorylase (E.C. 2.4.1.1. α-1, 4-glucan or the phosphate glucosyl transferase). Phosphorylase activity was determined by measurement of the rate of liberation of inorganic phosphate from glucose-1-phosphate in the presence of glycogen[8]. i.e. phosphorylase activity was measured in the direction of synthesis of polysaccharide. The chilled liver tissue was homogenized at 0 °C in 3 volume of 0.1 *M* NaF. Centrifuged at 1500 × *g* at 0 °C and the supernatant was used as the enzyme. Enzyme activity was assayed by the method of SUTHERLAND[9]. One unit of enzyme was defined as that amount which caused the liberation of 1.0 mg of inorganic

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Effect of allicin on certain liver enzymes of normal rats after 15 days oral administration (dose 100 mg/kg/day)

	Enzyme activity (Units/g tissue) α-Glucan phosphorylase	Glucose-6-phosphatase	Lipase
Normal rats	4.3 ± 0.10	6.5 ± 0.2	118.4 ± 8.5
Allicin treated rats	5.2 ± 0.15 [a]	5.7 ± 0.1 [a]	166.9 ± 9.1 [a]
Increase or decrease of enzyme activity (%)	+ 20.9	− 12.3	+ 41.0

Values are mean of 6 animals ± S.E. Units of activity expressed as indicated in the methods. Student's *t*-test. [a] $P < 0.01$, significantly different from the control values.

phosphorous under the conditions of experiment during 10 min. Enzyme activity was expressed as units per g of wet tissue.

Glucose-6-phosphatase (E.C. 3.1.3.9., D-glucose-6-phosphate phosphohydrolase). The method is based on the incubation of glucose-6-phosphate with the enzyme and determination of the liberated orthophosphate. The chilled liver tissue was homogenized at 0 °C with 0.1 *M* maleic acid buffer pH 6.5 (1:10 W/V) centrifuged at $1500 \times g$ at 0 °C and the supernatant was used as the enzyme preparation. Enzyme activity was assayed by the method described by SWANSON[10]. Enzyme activity is expressed as mg phosphorous liberated from the substrate under the conditions of the experiment by the enzyme present in 1 g tissue.

Lipase. (E.C. 3.1.1.3. glycerol ester hydrolase). Lipase activity was determined by measurement of the rate of liberation of fatty acid from olive oil following the titrimetric assay of serum lipase[11]. The chilled liver tissue was homogenized at 0 °C with 5 volume of 0.05 *M tris* buffer (pH 8) centrifuged at $1500 \times g$ at 0 °C and the supernatant was used as the enzyme preparation. Enzyme activity is expressed as μmol of free fatty acid liberated from the substrate under the conditions of the experiment by the enzyme derived from 1 g tissue.

Results. Allicin administration for a period of 15 days did not affect the normal fasting blood sugar level and the hexokinase activity of liver of normal rats. However α-glucan phosphorylase, glucose-6-phosphatase and lipase activities of liver after the drug administration were significantly affected; α-glucan phosphorylase and lipase activities increased significantly ($p < 0.01$) as compared to control values. These results are given in the Table.

Discussion. The low dose of allicin used in this experiment produced no significant change of blood sugar in normal rats, and this is different from the hypoglycemic action of a high dose of the same drug (0.25 g/kg) observed in alloxan diabetes[2]. The absence of a hypoglycemic action of allicin in normal rats may be due to the physiological maintenance of the normal blood sugar. The hypoglycemic activity of allicin in alloxan diabetes was explained on the basis of its action against insulin-inactivating-SH group compounds. The interaction of allicin with thiol groups[12,13] is well established and it may explain many of the therapeutic effects of allicin. As in the case of tolbutamide and insulin, allicin administration did not affect the hexokinase activity of liver of normal rats. According to the findings of TSUTOMU KITANI[14], tolbutamide and insulin had no in vivo effect on liver hexokinase in normal rats. However, these drugs have been reported to restore the lowered activity of α-glucan phosphorylase of liver to normal levels in experimental hyperglycemic rats[14]. The significant increase in α-glucan phosphorylase brought about by allicin in normal rats may be due to the enhancement of endogenous insulin action. Similarly the inhibitory effect of allicin on glucose-6-phosphatase can also be explained. LINKE et al.[15] observed that tolbutamide and insulin treatment lowered the glucose-6-phosphatase activity of liver of normal rats. On the basis of the present results, we may presume that allicin acts like the antidiabetic drugs on certain enzymes. However, the effects of these drugs on lipolysis and lipid levels are contradictory. Both insulin and tolbutamide are antilipolytic in vitro[16,17], but the marked hyperlipaemia which occured in diabetic patients on tolbutamide therapy has been reported to be corrected by insulin[18]. Therefore the in vivo effects of tolbutamide and insulin on lipolysis and lipid levels may not be similar. Such a difference is also seen in the effect of allicin on lipase. The significant enhancement of lipase activity following allicin administration can not be understood at present. The results presented in this paper throw some light on the therapeutic virtues of garlic which has been traditionally used by many patients suffering from diabetes and cardiac ailments[19,20].

Zusammenfassung. Nachweis, dass Allicin, während 15 Tagen verabreicht, gewisse Leberenzyme normaler Ratten in signifikanter Weise beeinflusst. So nahmen Phosphorylase- und Lipaseaktivität signifikant zu, während Glukose-6-Phosphatase vermindert wurde. Unverändert blieb die Aktivität von Hexokinase und der Blutzuckerspiegel; in gewisser Beziehung wirkte Allicin ähnlich wie Antidiabetica.

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