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EFFECT OF ACETOACETATE ON TYROSINE TRANSAMINASE AND TRYPTOPHAN OXYGENASE IN RAT LIVER

LIVER tyrosine transaminase (tyrosine aminotransferase E.C. 2.6.1.5) and tryptophan oxygenase (L-tryptophan oxygen oxidoreductase E.C. 1.13.1.12) activity can be induced by the injection of either insulin or cortisone¹⁻⁴. The activity of these enzymes were also reported to be increased in intact rat livers following the injection of sodium salt of acetoacetate⁵⁻⁶. It was not conclusive from these experiments whether increased activities of these enzymes were due to increased insulin secretion or to resulting stress and consequent secretion of corticoids. That ketone bodies injection results in transient and immediate rise in insulin secretion was demonstrated earlier from this laboratory⁷⁻⁸ and by others too⁹. Insulin content was, however, reported to be lowered after continuous injection of acetoacetate for a long period (2-3 months). Present work was undertaken to study the effect of acetoacetate on tyrosine transaminase and tryptophan oxygenase both in intact and adrenalectomized rats.

Female albino rats (100-125 gm body wt) maintained on stock laboratory diet were distributed into four groups of six each. Bilateral adrenalectomy was carried out for groups I and II rats. 0.9% saline was substituted for drinking water for adrenalectomized rats. The experiments were carried out one week after the adrenalectomy.

30 minutes before the sacrifice, groups II and IV rats were intraperitoneally injected with sodium salt of acetoacetate at a concentration of 300 mg/100 gm body wt. The sodium salt of acetoacetate was prepared by the method of Tidwell and Nagler¹⁰. Similar dose of sodium lactate was injected to groups I and III rats, which served as controls for groups II and IV respectively.

Rats were killed by decapitation, blood was collected in oxalated tubes for sugar estimation¹¹. Liver was removed, rinsed in ice cold water and blotted dry. A 10% homogenate was prepared in 0.25 M sucrose solution for tyrosine transaminase estimation and in 0.14 M KCl-0.002 NaOH for tryptophan oxygenase. Homogenates were centrifuged at 7,500 g for 30 minutes in refrigerated centrifuge at 0°. The supernatants were used as the enzyme sources. Tyrosine transaminase was estimated by the method of Rosen *et al.*² and tryptophan oxygenase was estimated by the method of Knox and Auerbach⁴. Protein content of the supernatant was determined by the usual biuret method.

Activity of tyrosine transaminase is increased 2 to 3 folds in intact as well as in adrenalectomized rats following the injection of acetoacetate as compared to controls. There was, however, no change in the activity of tryptophan oxygenase either in intact or adrenalectomized rats injected with acetoacetate as compared to their controls. Results are shown in Table I.

That acetoacetate injection results in an immediate rise in circulating insulin level is evident from the blood sugar level (Table I). Insulin is an inducer of tyrosine transaminase activity when injected to either intact or adrenalectomized rats¹⁻². Cortisone also induces tyrosine transaminase activity but takes longer time for maximum effect (five hours) as compared to one hour for insulin². It seems, therefore, that the observed increase in tyrosine transaminase activity in rats injected with acetoacetate is solely due to rise in circulating insulin level. Corticoids, therefore, are probably not responsible for the observed increase in tyrosine transaminase activity in our experiments.

Tryptophan oxygenase activity is unaffected when acetoacetate was injected to either intact or adrenalectomized rats within the experimental period of 30 minutes. However, this enzyme showed a 3 to 4 folds increase in its activity after five hours of acetoacetate injection to intact rats and no change in adrenalecto-

TABLE I
Effect of acetoacetate on the activities of tyrosine transaminase and tryptophan oxygenase in rat liver

Enzymes specific activity	Groups			
	III Normal + sodium lactate	IV Normal + acetoacetate	I Adrenalectomized + sodium lactate	II Adrenalectomized + acetoacetate
*Tyrosine transaminase ..	0.45 ± 0.08	1.25 ± 0.11	0.43 ± 0.05	1.20 ± 0.15
†Tryptophan oxygenase ..	0.015 ± 0.001	0.016 ± 0.011	0.013 ± 0.007	0.014 ± 0.009
Blood sugar level mg/100ml ..	65.00 ± 8.00	40.00 ± 8.00	60.00 ± 6.00	42.00 ± 5.00

Values are expressed as SEM ± of six rats. * Enzyme activity is expressed as micromoles of *p*-hydroxyphenylpyruvic acid formed /mg of protein/hr, under the assay conditions. † Enzyme activity is expressed as micromoles of kynurenine formed per hour under the assay conditions.

mized rats (unpublished data). The activity of tryptophan oxygenase (within 30 minutes) can be understood in the light of the fact that this enzyme requires longer time for its induction as shown by earlier workers^{4,12}.

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**ON THE OCCURRENCE OF
CHAROPHYTIC REMAINS FROM
THE INTERTRAPPEANS OF
GURMATKAL, GULBARGA DISTRICT,
MYSORE STATE**

PUBLISHED literature available on charophytic remains occurring in several parts of India relates to the work of the following investigators: Carter (1857) from Jabalpur; Rao and Rao (1939) from Rajahmundry; Rao and Sahni (1943) from Sausar; Mahadevan and Sharma (1948) from Vicarabad; Mitre (1952) from Rajmahal Hills; Rama Rao (1955) from

Belgaum, and Bhalla (1969) from Kateru intertrappeans. The present note deals with the occurrence of the charophytic remains from the intertrappeans of Gurumatkal. The identified taxa have been suitably illustrated.

Detailed geological mapping of the Gurmatkal area was undertaken by the author with a view to investigate the micro-fauna and flora. In this area the intertrappeans occur as isolated patches covering about 120 square miles. They vary in thickness from 10 to 40 feet, and extend in a linear pattern between the two basaltic flows. This lateral extension of the intertrappeans in different localities vary from 20 to 100 feet. They are chiefly made up of calcareous marls, reddish-brown shales with thin veins of gypsum, cherts and flints. The lower basaltic flow rests unconformably over the granites and gneisses (Archaean). The cherts and flints are tuffaceous, in which are preserved the charophytic remains in large numbers. Thickness of this chara bearing bed varies from 1 to 4 feet. In most of the outcrops examined, cherts invariably occupy the uppermost position. Soft sediments such as marls and reddish-brown shales required no special treatment and all the samples yielded quite large number of well-preserved micro-fossils belonging to different groups—ostracodes, gasteropods and lamelli-branches. However, the harder sediments namely tuffaceous cherts and flints had to be specially treated with hydrogen peroxide before they were subjected to repeated freezing and thawing processes. Well-preserved specimens were exclusively recovered from the Suddogu Halla section. (Topo sheet No. 56 H/NW. 77° 23' 30", 16° 52' 0").

These rich charophytic remains consist of perfectly preserved Oogonia (fruits) or the Nucules. Scanty impressional fossils of chara vegetative parts have also been preserved. This assemblage includes 8 species (Taxa), 2 of