Studies on Glutamic-Oxalacetic (GOT) and Glutamic-Pyruvic (GPT) Transaminases of Swine Kidney Worm Stephanurus dentatus (Diesing, 1839)

I. Assay and General Properties

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Summary. Biochemical studies on the two transaminases GOT and GPT of swine kidney worm Stephanurus dentatus have been made. GOT has been found much more active than GPT. Enzyme activities are based on the formation of oxaloacetate (GOT) or pyruvate (GPT) from aspartic acid and alanine respectively with oxoglutarate. A linear relationship is observed between the enzyme concentration and activity. GOT shows a maximum activity at pH 8.0 and Michaelis constant 9 × 10⁻³ M for male and 2.9 × 10⁻³ M for female. GPT has an optimum pH of 7.5 and a Michaelis constant 19 × 10⁻³ M for male and 8 × 10⁻³ M for female. The optimum temperature for both GOT and GPT was 60° C.

Introduction

The transaminases which form a link between the metabolism of amino acids, lipids and carbohydrates have been extensively studied in mammalian tissues, microorganisms and plants etc. (Cohen, 1961). But very little work has been done on parasitic nematodes. The data available are on Ascaris lumbricoides (Cavier and Savel, 1954; Savel, 1955; Pollak and Fairbairn, 1955), and Ascaridia galli (Lestan et al., 1974). Despite the veterinary importance of Stephanurus dentatus, which occurs frequently in kidney, peritoneal fat and pelvis of swine, comparatively very little is known of its enzyme systems. The present study is concerned with the biochemical characterization of two transaminases, glutamic-oxalacetic (GOT) and glutamic-pyruvic (GPT), of S. dentatus.

Materials and Methods

Adult worms were collected from the peritoneal fat and pelvis of kidney of pigs slaughtered locally. They were placed immediately in a cold normal saline and washed repeatedly. Homogenates were prepared in ice cold normal saline solution (2% w/v) with a ground glass homogenizer. The crude homogenates were centrifuged at 0° C at 2000 g for 15 min. The supernatant fluid was stored under toluene in a refrigerator and used as source of enzyme for assay as required.

The enzyme activity was estimated according to Reitman and Frankel (1957) using Spectronic-20 spectrophotometer. The results are expressed in RF-units/mg wet weight. One unit of enzyme activity is equivalent to the formation of 4.82 × 10⁻⁴ µM of glutamate/min. All the experiments were run in
duplicate and repeated at least three times with the different homogenates prepared separately. In all cases appropriate controls were run simultaneously. A pH of 7.5 for GOT and 8.0 for GPT was used for evaluation of kinetic constants. All the chemicals used were obtained from Sigma Chemical Company, U.S.A.

The points in the figures represent the average of five readings.

Results

Optimum pH. As shown in Figure 1, the GOT and GPT activity was determined over a pH range of 4.5 to 10.0. In males GOT activity ranged from 12–20 (16 ± 2.3) and GPT from 8–14 (10 ± 1.7) RF-units/mg wet weight. In females, however, transamination reaction was not very strong and GOT activity ranged from 6–15 (10.0 ± 2.0) and GPT from 3–10 (6.0 ± 1.4) RF-units/mg wet weight. The maximum activity in male and female homogenates was recorded at pH 8.0 for GOT and pH 7.5 for GPT respectively at a temperature of 37° C. On either side of these values there was a gradual fall in the activities of both the enzymes. No activity was observed below pH 5.0 and 6.0 in GOT and GPT respectively.

Substrate Concentration. The effect of substrate concentration on GOT and GPT reaction velocities measured at 37° C is expressed in Figures 2 and 3 respectively. Both the enzymes show a more or less proportional increase in the activity with the corresponding increase in substrate up to a final concentration of 10 mM for GOT and 14 mM for GPT. Michaelis constants for the two substrate systems (aspartic acid + α-ketoglutaric acid) and (alanine + α-ketoglutaric acid) were computed by the method of Lineweaver and Burk (1934) and the Km values of GOT and GPT were