Analysis of Aminotransferase in Liver and Serum by Gel Filtration

The tissue aminotransferase, which was mainly found in mitochondrial as well as in cell sap fraction, was characterized by several methods, such as electrophoresis, enzyme kinetics, anion exchange chromatography and immunochemistry[1-9]. The serum aminotransferase was also analysed by electrophoresis or immunochemistry[10-14], suggesting its cytoplasmic origin. To clarify the exact nature of serum aminotransferase, especially under pathological conditions, the present report attempts to analyse serum and liver aminotransferase by gel filtration.

The liver obtained from male Wistar strain rats was perfused and homogenized in 0.25 *M* sucrose, followed as an isolation of mitochondrial and supernatant fraction by the suitable centrifugation[15]. The mitochondrial fraction, washed and suspended in 0.25 *M* sucrose, was exposed for 10 min to 20 KC/sec in a sonic oscillator at 0°–4 °C. After 10 min the suspension was centrifuged for 10 min at 10,000 *g* and the supernatant was obtained[14]. The serums were obtained from rats, 12 and 24 h after an i.p. injection of carbon tetrachloride, 0.015 ml/100 g body weight, and from the patient with acute infectious hepatitis.

The rat liver fractions and serums were subjected to the gel filtration[16] using a 2.2 · 80 cm Sephadex G-200 column in cold room. For elution, buffered saline (0.01 *M* phosphate buffer, pH 7.2 in physiological saline) was used. The effluent was collected in each 4.5 ml portion. The typical flow rate was 10 ml, 2 ml and 1 ml/h for serum, the liver supernatant fraction and the soluble mitochondrial fraction respectively. The molecular weight of aminotransferase was calculated from the void volume (V_0) and the effluent volume (V) by SQUIRE's formula[17]: $M^{1/3} = 73.0/0.480 \cdot [1.480 - (V/V_0)^{1/3}]$. Aspartate and alanine aminotransferase activity[18], which is referred to

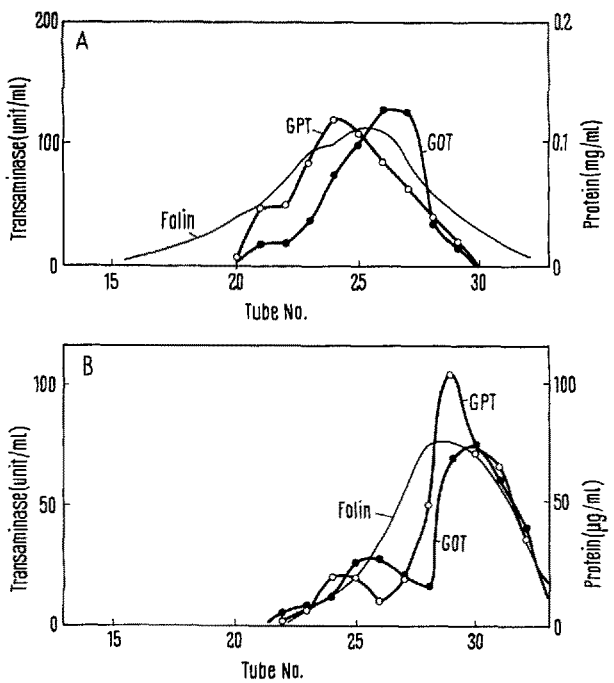

Fig. 1. Gel filtration of aminotransferase activity in supernatant fraction (A) and mitochondrial fraction (B) from rat liver.

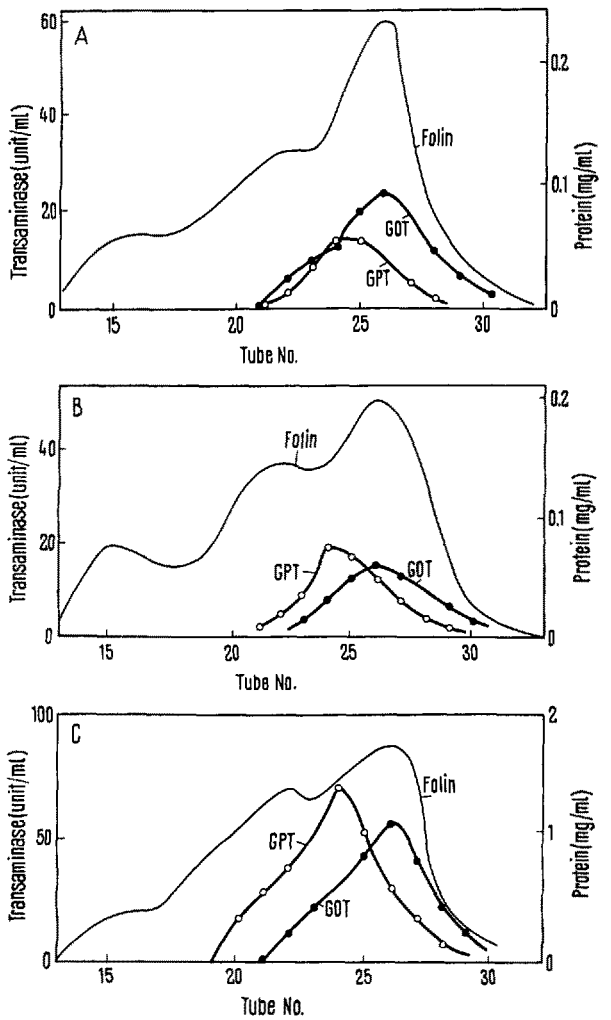

Fig. 2. Gel filtration of aminotransferase activity in serums; rat serum, 12 h (A) and 24 h (B) after carbon tetrachloride intoxication and a human hepatitis serum (C).

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as GOT and GPT respectively, and protein content[19] were determined on the fractionated portion. The enzyme activity was expressed as Karmen unit.

The elution diagrams of the supernatant and the soluble mitochondrial fraction from the rat liver are illustrated in Figure 1. The highest activity of the mitochondrial GOT (mGOT) and GPT (mGPT) was found in tube 29 and tube 28, whereas the supernatant GOT (sGOT) and GPT (sGPT) were recovered in tube 26 and tube 24 respectively. The results were fairly reproducible. The faster effluent portion of mGOT and mGPT may be due to the contamination of sGOT and sGPT. The effluent volume for the maximum activity of mGOT, mGPT, sGOT and sGPT was 130 ml, 126 ml, 117 ml and 108 ml respectively. Because the void volume was 74 ml, the range of the calculated molecular weight of mGOT, sGOT, mGPT and sGPT was 77–72, 115–120, 86–90 and 140–150 · 10^3 respectively. After the gel filtration, the recovery of GOT and GPT was 6–20%.

The elution diagrams of serums from the rat with carbon tetrachloride intoxication and a patient with infectious hepatitis are represented in Figure 2. The highest activity of GOT and GPT was found in tube 26 and 24 respectively, which correspond to a molecular weight of 115–120 and 140–150 · 10^3. The recovery of the activity was 32–93%.

The molecular weight of mGOT and sGOT, as calculated in the present report coincide with the molecular weight of crystallized GOT from beef liver[7]. The molecular weight of serum aminotransferase, which has not been elucidated so far, coincides fairly with sGOT and sGPT, but not with mGOT and mGPT. These results suggest that the elevated aminotransferase activity in serums with liver injury originates mainly from the supernatant fraction of the liver, as has been assumed from other evidence[10–13]. The present report, however, does not exclude the possibility of a release of mGOT and mGPT, which have a very short intravascular half-life[11].

Zusammenfassung. Mit der Gel-Infiltrationsanalyse von Serum und Leberfraktion normaler und CCl_4-exponierter Ratten wurde die Herkunft der Serum-Aminotransferase untersucht, was zur Annahme führte, dass seine Aktivitätssteigerung bei der Leberschädigung von der überstehenden Leberfraktion abhängt.

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Effects of Dehydration on Rat's Hypothalamic Acid-Phosphatase

A strong acid phosphatase activity has been demonstrated hystochemically in the hypothalamic neurosecretory cells of several mammals[1–3], including the rat[4,5]; a further increase in this activity takes place after submitting the hypothalamo-hypophyseal system to functional demands[6,7]. It was considered of interest to assess quantitatively this variation in the hypothalamus of dehydrated rats as an increase seems to take place in the pars nervosa after dehydration in the sparrow[8].

Acid phosphatase was estimated quantitatively in hypothalamic samples containing the magnocellular neurosecretory nuclei (supraoptic, paraventricular, accessory supraoptic) and median eminence of rats dehydrated by withholding water intake during 7 days. Control animals were allowed ad libitum drinking. The hypothalamus was defined by making incisions 3 mm deep just rostrally to the optic chiasm and mammillary bodies and along the lateral borders of the tuber cinereum.

Phosphatase activity was estimated by a procedure similar to the one used by Kobayashi and Farner[9]. Enzymatic activity is expressed in micrograms of para-Nitrophenol liberated per mg of wet tissue at 37 ± 0.2 °C in 45 min. At pH 5.4 phosphatase activity was a linear function of the enzyme concentration, both in the region of the supraoptic nucleus and median eminence. All determinations were controlled with blanks processed in identical fashion as the test tubes, except for the incubation period.

The results in the Table indicate that acute dehydration significantly increases acid-phosphatase activity in the hypothalamic zone containing the magnocellular neurosecretory neurons, the axons of paraventricular cells and the neurohemal structures of the median eminence, thus confirming previous histochemical findings. A similar increase in phosphomonoesterase activity has been reported

Effect of dehydration on rat's hypothalamic acid phosphatase

Condition	Activity/mg[a]	*P* Value
Normal	6.96 ± 0.14 (8)[b]	> 0.001
Dehydrated	8.00 ± 0.20 (7)	

[a] Phosphatase activity is expressed in μg of *p*-nitrophenol/mg wet tissue liberated in 45 min. [b] Mean ± S.E.; No. of animals is indicated in parentheses.

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