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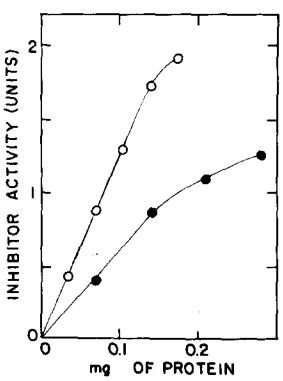
Increased activity of deoxyribonuclease inhibitor in rat serum after partial hepatectomy

The inhibitor of deoxyribonuclease in tissues of animal origin have been described by several investigators[1-3]. Recently, LINDBERG[4] has succeeded in crystallizing two closely related inhibitors from calf spleen to act on pancreatic deoxyribonuclease (deoxyribonucleate oligonucleotidohydrolase, EC 3.1.4.5). The presence of deoxyribonuclease inhibitor in rat serum was first described by OKADA *et al.*[5], and BERGER AND MAY[6] have recently purified the inhibitor from rat serum and shown it to have close similarity to calf spleen inhibitor II extracted by LINDBERG[4].

This paper describes an investigation of the finding that partial hepatectomy caused an increase in the activity of deoxyribonuclease inhibitor in serum. Surgical removal under ether anesthesia was carried out as described by HIGGINS AND ANDERSON[7] on male Wistar rats weighing 80–100 g. Blood samples were taken by heart puncture under ether anesthesisa, and the serum was separated by centrifugation. In assaying deoxyribonuclease inhibitor, the serum was dialyzed against 0.01 M Tris buffer (pH 7.5) at 4° overnight.

Inhibitor was assayed by estimating the inhibition of pancreatic deoxyribonuclease by the dialyzed rat serum. A unit of inhibitor activity was defined as that causing 50 % inhibition of 0.02 μg crystalline pancreatic deoxyribonuclease under assay conditions.

As shown in Fig. 1, partial hepatectomy caused an increase in the activity of deoxyribonuclease inhibitor in serum. Increased activity remained the same at 24 h after the operation.



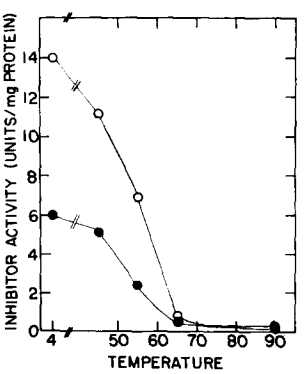


Fig. 1. Inhibition of deoxyribonuclease activity in serum from normal and partially hepatectomized rats. Inhibitor activity was measured in the dialyzed serum from normal and partially hepatectomized rats at 18 h after the operation. Assays were carried out in a total volumes of 0.3 ml containing: 30 μmoles Tris–HCl buffer (pH 7.5), 2.5 μmoles $MgCl_2$, 300 μg of native calf thymus DNA, 0.02 μg of crystalline pancreatic deoxyribonuclease, and dialyzed serum. The reaction was stopped with 3 ml of 5 % $HClO_4$. The absorbance of the acid supernatant was determined at 260 mμ. ●, serum from normal rats; ○, serum from partially hepatectomized rats.

Fig. 2. Thermal stability of deoxyribonuclease inhibitor in serum from normal and partially hepatectomized rats. The activity of pancreatic deoxyribonuclease was assayed by adding the inhibitor preparation (70 μg of protein) after heating for 5 min in 0.01 M Tris–HCl buffer (pH 7.5) at the indicated temperature. Assay conditions are described in Fig. 1. ●, serum from normal rats; ○, serum from partially hepatectomized rats.

The thermal stability of the inhibitor was determined by assaying the activity remaining after incubation for 5 min at various temperatures between 45 and 90° in 0.01 M Tris buffer (pH 7.5). The results shown in Fig. 2 indicate that the activity of the inhibitor protein was almost completely destroyed at 60°. The inhibitor activities in normal and partially hepatectomized animals were indistinguishably affected by heat.

Similar observations have been reported for ribonuclease inhibitor, that the activity of ribonuclease inhibitor was found to be higher in serum after partial hepatectomy than in serum from normal rats[8]. Whether increased activity in deoxyribonuclease inhibitor is due to *de novo* synthesis or to activation is not known, though the former seems more likely in view of the results that increased activity may be responsible for the increased protein synthesis[8,9]. It is possible that this inhibitor may actually control the activity of deoxyribonucleases. We are now attempting to find the function of deoxyribonuclease inhibitor in the overall control of DNA synthesis.

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1 G. SIEBERT, R. LANG, S. LUCINS-LANG, Z. HERBERT, G. STARK, G. ROSSMULLER AND H. JOCKEL, *Z. Physiol. Chem.*, 295 (1953) 229.
2 W. FRISCH-NIGGEMEYER AND O. HOFFMANN-OSTENHOF, *Monatsh.*, 81 (1950) 607.
3 L. M. GILBERT, W. G. OVEREND AND M. WEBB, *Exptl. Cell Res.*, 2 (1951) 349.
4 M. L. LINDBERG, *J. Biol. Chem.*, 241 (1966) 1246.
5 S. OKADA, E. R. GORDON, R. KING AND L. H. HEMPELMANN, *Arch. Biochem. Biophys.* 70 (1957) 469.
6 G. BERGER AND P. MAY, *Biochim. Biophys. Acra*, 139 (1967) 148.
7 C. M. HIGGINS AND R. M. ANDERSON, *Arch. Pathol.*, 12 (1931) 186.
8 K. TSUKADA, *Biochim. Biophys. Acta*, 186 (1969) 21.
9 K. TSUKADA, T. MORIYAMA, O. DOI AND I. LIEBERMAN, *J. Biol. Chem.*, 243 (1968) 1152.

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