〔J. Clin. Biochem. Nutr., **2**, 179 (1987)〕

Establishment of an Improved Enzyme Immunoassay for Urinary Human Epidermal Growth Factor/Urogastrone.

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A rapid two-site enzyme immunoassay (EIA) for human epidemal growth factor (hEGF) was developed. Our rapid EIA was based on the sandwiching of an antigen between anti-hEGF IgG-coated polystyrene beads and anti-hEGF Fab'-linked peroxidase (horseradish peroxidase, EC. 1. 11. 1. 7). This method is so rapid that we can get the results within 5 h. The reproducibility of within- and betweenassay was 4.8 to 7.1% and 3.5 to 7.2%, respectively. And discriminatory sensitivity was as low as 5pg/ml. The recovery of hEGF from urine and serum by this method was approximately 100%, and the serial dilution curves of unextracted human urine and serum samples were parellel with that of standard hEGF (Urogastrone). We applied this method to assay of hEGF content in urine of patients with a variety of tumors.

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Studies on Production of Human Epidermal Growth Factor and Its Receptor in Human Hepatoma Cell Line (PLC/PRF/5).

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Among cell growth factors, epidermal growth factor (EGF), which was originally isolated from the submaxillary glands of male mice and subsequently from human urine, acts biologically to stimulate cell proliferation in epidermal and epithelial tissues in animal and various nontransformed and transformed cell types in cell culture. We studied on the role of EGF on the production and its receptor in human hepatoma cell line. As a result, it was demonstrated that Ax cells synthesized EGF and contained the EGF-receptor on the cell membranes. These results suggest that there is a possibility of the contribution to the autocrine/paracrine mechanism in the growth of Ax cells.

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Primary Structure of α-Bungarotoxin --- Six Amino Acino Residues Differ from the Previously Reported Sequence.

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Venoms from snakes belonging to the families Elapidae (cobra, mumbas, tiger snakes, black snakes, taipan, etc.) and Hydrophiidae (sea Snakes) are highly toxic and produce flaccid paralysis and respiratory failure in animals. α-Bungarotoxin(α-BuTx) was isolated from the venom of the Formosan banded krait (*Bungarus multicinctus*). The amino acid sequence was determined by a combination of conventional methods. In contrast to the sequence of α-BuTx reported by Mebs *et al.* 〔Biochem. Biophys. Res. Commun., 44, 711 (1971)〕, our results revealed the presence of Ser-Pro-Ile, Pro-His and Gln-Arg at positions 9-11, 67-68 and 71-72 from the amino-terminal, respectively, and not Ile-Pro-Ser, His-Pro and Arg-Gln as reported previously.