SUMMARY

Glycosaminoglycans are charged polyanions composed of repeating disaccharide units and are important component of extracellular matrix. Eight GAG's has been studied in detail. Except hyaluronate all other GAG's e.g., chondroitin sulphate, heparin, keratan sulphate are found as proteoglycan in vivo. Almost all mammalian cells contains hyaluronate. It is an unbranched helical polymer, composed of repeat disaccharide units of N-acetyl D-glucosamine and B-D-glucuronic acid. Hyaluronic acid has many important role in different biological processes e.g., cell adhesion, cell aggration. The role of hyaluronate has been extensively studied at discrete stages of early development of tissues undergoing striking morphogenesis.

Recently, numerous studies have shown that hyaluronate are associated with the surface of many different type of cells and their levels vary under different physiological conditions. Though the evidence of hyaluronic interaction with the cell surface reported earlier, the first report of hyaluronic acid binding protein, hyaluronectin came from Delpech et al. in 1981. Later, the same group reported the presence of hyaluronectin in tumor heterografts from nude mice. The extensive documentation of biological significance of hyaluronic acid on one side and recent discovery of hyaluronic acid binding protein on other side provoked us to purify and characterize hyaluronic acid binding protein from normal rat brain and liver.

We purified HBP from adult rat brain for the first time using hyaluronate-sepharose affinity columns. Hyaluronate-sepharose column was prepared according to Cambaiso's method.
The homogeneity of protein was checked by 7.5% PAGE according to Davis\(^3\). The molecular weight of HBP as determined on gel chromatography using G-100 was found to be 68,000 daltons. On SDS-PAGE according to Laemmli\(^4\), the protein was found to have single sub-unit of 13,500. Thus, the protein was composed of five identical subunits.

Furthermore for the characterisation of HBP, the purified enzyme was assayed for the activity of hyaluronidase\(^5\). It was found that purified HBP is not an enzyme hyaluronidase Sialic acid content\(^6\) were measured and found to 0.285 mmoles/mg of HBP indicating the nature of this protein as glycoprotein.

Amino acid analysis of HBP was done according to the method of Moore \textit{et al.}\(^7\), modified by Chatterjee and it was found to be glycine glutamic acid rich protein. Amino acid composition of HBP confirmed that it is a different protein from fibronectin, gelatin binding protein and link protein although they are known to bind HA.

Purified HBP was further found to be protease sensitive but resistant to chondroitinase ABC suggesting that no GAGs are linked with HBP. Binding of HBP to HA immobilized on affinity column were identical at 4\(^\circ\)C and 37\(^\circ\)C. Recently, Turley\(^8\) reported the partial purification of HA binding protein from media of chick fibroblast. The molecular weight of their partial purified protein is close to that of ours in the range of 60,000-63,000 daltons. Moreover, the procedure reported by us is much simpler.

The successful purification on HBP from rat brain led us to several questions. Firstly, we were interested
know the specificity of purified HBP to GAGs other than hyaluronate and also to the hydrolysis products of HA, i.e., N-acetyl-glucosamine and glucuronic acid. Secondly, to discover the possibilities, if any, about the tissue specificity of this purified HBP and finally, to evaluate its physiological role.

The affinity of HBP was examined by prior incubation of HBP to HA, heparin, glucuronic acid and N-acetyl-glucosamine and loading on HA-sepharose affinity columns. It was seen that HBP bound specifically to HA and the binding of HBP to HA on immobilized sepharose column was blocked by pretreatment of HBP with HA. In addition, the affinity of HBP to heparin and chondroitin-4-sulphate was also checked by using heparin-sepharose and chondroitin-4-sulphate-sepharose affinity columns. It was observed that HBP did not bind to heparin or chondroitin sulphate, suggesting its specificity to HA only. Since HBP bound to HA and free HBP are expected to differ in their molecular weight, we were able to distinguish two peaks in Sephadex G-100 and Sephadex G-200 column. One of which was free HBP and other was HBP bound with HA. The level of HA was estimated in all the fractions and HA association with HBP was confirmed. Furthermore, the HBP-HA complex was shown on 7.5% polyacrylamide gel where migration of the complex was different from that of purified HBP and free HA.

Since there is no availability of commercial radioactive HA we labelled pure HBP with $^{125}$I by the method of Fraker et al. and iodinated HBP was used for binding test with HA. The HBP complex with HA was precipitated with 7.5% PEG whereas, free HBP remained in the supernatant. More counts was recovered in PEG precipitate in conjugation
with 40 ng of HA. In the control tube containing iodinated HBP alone, PEG addition allowed to precipitate but most of the counts were recovered in the supernatant only. Thus our data indicate the binding of HA with HBP.

As the preceding reports gave a detailed account of purification of HBP and its possible characterisation for the first time in rat brain, our further programme of study was to evaluate the levels of HBP in liver. Liver is the seat of various glycopeptides and also the sulphation of GAGs takes place in liver. We were inquisitive to know the levels of HBP in adult rats.

The HBP purified from rat liver was similar as that reported from rat brain. All the methods used for the characterisation of this liver HBP was also similar as reported earlier in brain. The molecular weight of protein on sephadex G-100 was found to be 64,000. The difference in molecular weights of HBP from liver and brain may be due to glycoprotein nature of the protein.

The liver HBP, like brain HBP was also found to have single subunit on SDS-PAGE. The molecular weight of this sub-unit was found to be 12,000. Thus, the liver HBP was in close resemblance to brain HBP as the migration of HBP from both sources moved same distance on 7.5% polyacrylamide gels, thus proving its homogeneity and similarity. Both the proteins possessed single sub-unit on SDS-PAGE and sialic acid content was present in both purified HBP from liver and brain, though slight variations were found in their content. In this context it's interesting to add the reports of Underhill purification of antigen which binds to hyaluronate from horse serum and that of Turley
the partially purified HA-binding protein from media of chick fibroblasts. Both the HA binding proteins were reported to be specific for hyaluronate.

Furthermore it was found that HBP was widely distributed in tissues like kidney, liver, brain, heart and eye of neo-natal rat and the highest levels of HBP were found in kidney, followed by liver and brain. The total HA content in tissues of neo-natal rat, like kidney, liver, brain, heart and eye were found to have inverse relationship with levels of HBP from the same tissues. Finally, the last part of our study, once again reveals a fascinating results of HBP levels in aging rats. The levels of HBP concomitantly decreased with aging. Thus, it is suggested that HBP may have some physiological importance as it is involved in the development.

The homogeneous purification and characterization of hyaluronic acid binding protein from rat brain and liver will help us in future to study the mechanism of the action of hyaluronic acid in different biological processes. The availability of pure HBP may be utilized to adopt a procedure for determination of HA, which will be highly specific, rapid sensitive and easy compared to the existing primitive procedure.