These results demonstrate that insulin a well-known hypoglycemic hormone was capable of stimulating the production of maspin through the increased synthesis of NO both in neutrophils and in breast tissue homogenate. Furthermore it was also found that not only the insulin induced synthesis of NO and maspin production was severely impaired in malignant breast cancer tissue itself but similar impairment of both NO and maspin production was also found in the case of apparently nonmalignant neutrophils in breast cancer. These results indicated that the synthesis of the anti-tumor protein in both malignant and nonmalignant cells in this condition was severely affected. Although the details of the pathological mechanism responsible for the impaired NO synthesis not known in breast cancer at present, these results nevertheless indicated a key physiological role of NO in the systemic synthesis of maspin. Furthermore, since the insulin receptors on the cell surface are critically important in the synthesis of NO leading to the maspin production, any decrease in the hormone receptor numbers would result in the decreased maspin synthesis in the cell. As present above, even the nonmalignant neutrophils displayed impairment of insulin induced maspin production in breast cancer (Fig. 4). The apparent contradiction that even in non-malignant cells the insulin induced maspin production is impaired could be explained by the marked reduction of insulin receptor numbers on the neutrophil surface in breast cancer (Fig. 6). The decrease of cell surface insulin receptor number is not unique for neutrophils in breast cancer. We have reported before that the hormone receptor numbers in non-malignant erythrocytes are also markedly decreased in this condition (Chakraborty et al. 2004). As such it is possible that the decrease of insulin receptor number in the cell surface is a manifestation of systemic flaw in the receptor protein synthesis in general in breast cancer. The defective insulin receptor synthesis in breast cancer may also explain the occurrence of diabetes mellitus in this condition. (Bruning et al. 1992, Weiderpass et al. 1997). The effect of insulin on the production of maspin through the synthesis of NO also indicated a novel role of this well-known antidiabetic hormone in the prevention of breast cancer. Furthermore the treatment of cells with PGE₁ increased the available insulin receptor numbers not only in normal condition but also in the case of breast cancer (Fig. 6). These results indicated that although PGE₁ itself does not increase maspin production, its presence in the system could be beneficial for the insulin
induced maspin production in breast cancer. As described above insulin was capable of stimulating maspin synthesis in normal neutrophils which are well known for their protection of the host against infection, indicated that these cells might also be involved in the protection of the host against the invasion of breast cancer in the presence of insulin. The protective effect of neutrophils through insulin induced maspin release may not be limited to breast cancer alone since maspin has been reported to control other kinds of cancer like oral squamous cell carcinoma, prostate cancer (Xia et al. 2000; Shi et al. 2001; Li et al. 2005).

It was thought that the effect of insulin in neutrophils in breast cancer was related to the nonmalignant nature of these cells in the circulation. However the degree of restoration of the impaired NO and maspin by insulin in the presence of PGE₁ in malignant breast cancer tissue homogenate was found to be greater than that in non malignant neutrophils indicating that the non malignant nature of the neutrophils perse was not responsible for the observed impairment of insulin effects. It could be argued that the insulin-induced release of maspin from the neutrophils may not be of physiologic importance due to the possibility of exhaustion of the cellular pool of the preformed protein. On the other hand it is known that the matured neutrophils, after their release from the bone marrow, circulate about 6 h in the circulation and after this period the old neutrophils replaced by newly released neutrophils (Golde and Cline. 1977). Due to the rapid turnover of these cells in the circulation it is possible that the insulin induced production of maspin from neutrophils could have a significant role in the steady supply of the anti-tumor protein in the system. The effect of maspin produced by insulin through the production of NO, could also be directly shown by treating these cells with NO solution or by using non physiological sodium nitroprusside, a NO generating agent in situ (Lorenzo et al. 1971) in the reaction mixture (Table-3).

Finally the increase of maspin production in neutrophils may not be due to the release of maspin from the cell membranes and extracellular matrix (Pemberton et al. 1997). In a preliminary study we have found that the increase of cellular NO level by insulin, aspirin or by NO itself increased maspin synthesis in neutrophils through increased maspin -mRNA synthesis within 60 min at 37°C (Table-3). These results further supported our conclusion that the stimulation of cellular synthesis of maspin was induced by NO itself, but not by the stimulators of NO synthesis themselves.

Furthermore these results also demonstrated that the stimulation of NO synthesis either
by aspirin or by insulin resulted in the stimulation of maspin synthesis with cellular increase of cyclic GMP level (Table-1).

Since various biological effects of NO are known to be mediated through the increase of cyclic GMP level (Arnold et al. 1977) it is then possible that the impaired expression of maspin in breast cancer cells was a pathological consequence of impaired cellular availability of cyclic GMP.

Our results indicated that the systemic increase of insulin to normal postprandial ranges, which is also known to stimulate endothelial PGI$_2$ synthesis (Kahn & Sinha.1990) might “normalize” maspin production in breast cancer. Alternatively, ingestion of low amounts of aspirin might help to control the development of breast cancer through increased maspin production in this condition through the synthesis of NO (Girish et al. 2006).