Also of interest is the slight, but statistically significant increase in CCK plasma levels after intragastric administration of a 10% saccharose solution. The increases in plasma CCK after the 3 nutrients cannot be explained by distention of the proximal small intestine as the control group with distilled water showed no effect on CCK levels.

It has been proposed that a negative feedback mechanism regulates pancreatic secretion in rats. Intraluminal trypsin in the proximal small intestine is assumed to play a central role in this mechanism, presumably by inhibiting CCK release, resulting in a decrease in pancreatic enzyme secretion [14]. The finding that feeding of trypsin inhibitors causes a significant and sustained increase in plasma CCK levels and thereby an increase in pancreatic secretion supports this hypothesis [12, 14, 16].

Our study demonstrates that, although intraluminal trypsin activity may play an important role in the regulation of pancreatic enzyme secretion, other mechanisms of CCK release do play a role in the postprandial stimulation of the pancreas in rats. Triglycerides and oligosaccharides give rise to a plasma CCK increment above the threshold for stimulation of pancreatic secretion, even though they do not possess any trypsin inhibiting activity as casein does [15].

The results of the present study lead to the following conclusions: 1) Casein, an unhydrolyzed protein, is a potent stimulus for CCK release in the rat. 2) Triglycerides, although less potent, still produce a CCK increment above the threshold for stimulation of pancreatic secretion. 3) Oligosaccharides also produce a CCK increase above this threshold. 4) These effects are not due to distention of the stomach or proximal small intestine.

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Influence of age on epidermal growth factor receptor level in the rat brain

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Summary. The influence of age on ^{125}I-epidermal growth factor (EGF) binding to rat brain plasma membranes was investigated. The specific binding of EGF to membranes decreased gradually with age in both male and female rats. There was no significant difference in the specific binding between males and females. Scatchard analysis of the binding data showed that the decrease in EGF binding with age was due to a decrease in the number of EGF receptors.
Key words. Age; epidermal growth factor receptor; brain; rat.

Epidermal growth factor (EGF) is a single chain polypeptide of 53 amino acids, first isolated from male mouse submandibular gland [1] and subsequently from human urine [2]. This peptide is a potent mitogen and differentiation factor for a variety of cells both in vivo and in vitro . Like other polypeptide hormones, EGF binds to a specific plasma membrane receptor in target cells [4]. It has been generally accepted that one of the earliest signals of EGF action is the autophosphorylation of EGF receptor following the activation of protein kinase integrated in the receptor molecule [5]. The EGF receptor is known to be distributed in various organs including the liver, skin, and gastrointestinal tract [4,6]. Moreover, a recent report has demonstrated the presence of EGF receptor in the brain of rabbits [7]. In this study, we investigated the influence of age on EGF receptor level in the rat brain.

Materials and methods. EGF was isolated from male mouse submandibular glands by the method of Savage and Cohen [8]. ^{125}I-EGF was purchased from New England Nuclear, Boston, MA. Phenylmethylsulfonyl fluoride (PMSF) and leupeptin were purchased from Sigma Chemical Co., St. Louis, MO.

Male and female Wistar rats of different ages were killed by ether anesthesia, and their whole brains were removed. The brain from each animal was homogenized in an all-glass homogenizer with 30 vol of 1 mM $NaHCO_3$ containing 2 mM PMSF and 10 μg/ml leupeptin. The homogenate was centrifuged for 10 min at 600 × g, and the supernatant obtained was then centrifuged for 30 min at 20,000 × g. The resulting pellet was washed once with the 1 mM $NaHCO_3$ solution described above, suspended in the incubation buffer (100 mM HEPES, pH 7.4, containing 120 mM NaCl, 1.2 mM $MgSO_4$, 2.5 mM KCl, 15 mM $NaC_2H_3O_2$, 10 mM glucose, and 1 mM EDTA), and used as the membrane preparation. Protein content in the sample was measured by the method of Lowry et al. [9].

The components of the binding assay consisted of ^{125}I-EGF (1 or 4 nM) and membrane preparation (500 μg protein) in 0.15 ml of the incubation buffer containing 1% bovine serum albumin (BSA). The medium was incubated at 24 °C for 90 min with or without an excess of unlabeled EGF (2000 nM), and then centrifuged for 5 min at 20,000 × g. The resulting pellet was washed once with 0.5 ml of the incubation buffer containing 1% BSA, and radioactivity in the

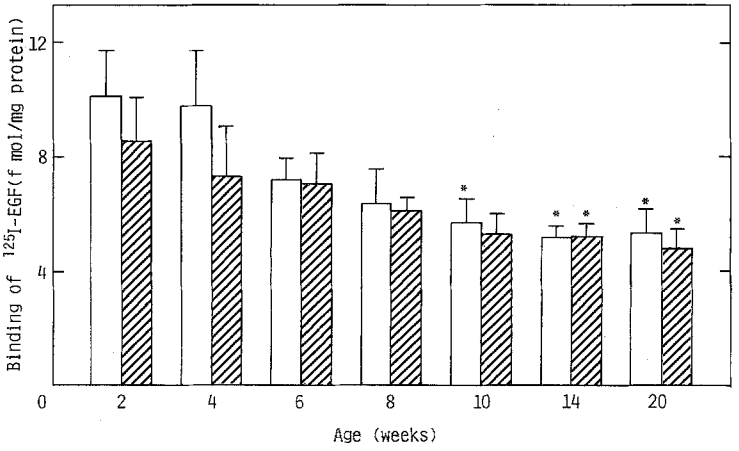

Figure 1. Binding of EGF to brain membranes from male (□) and female (▨) rats of different ages. Membranes (500 µg protein) were incubated with 4 nM ^{125}I-EGF in the presence or absence of 2000 nM unlabeled EGF, and specific binding was determined. Each column shows the mean ± SE of 6 animals. *$p < 0.05$ as compared with the value of 2-week-old rats.

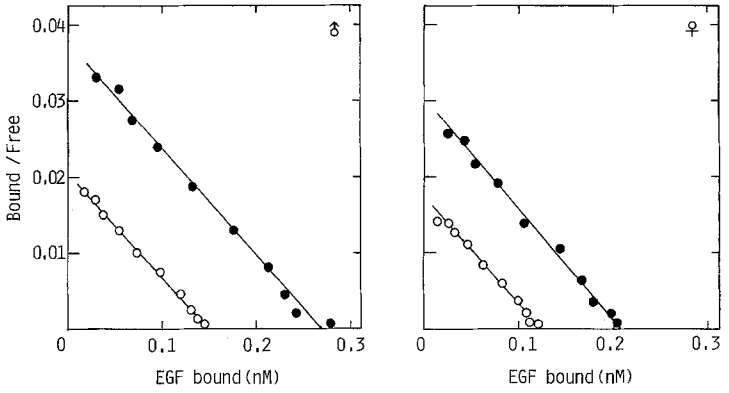

Figure 2. Scatchard plots of EGF binding to brain membranes from male and female rats of 2 weeks (●) and 20 weeks (○) of age. Membranes (500 µg protein) were incubated with 1 nM ^{125}I-EGF in the presence of various concentrations of unlabeled EGF (0 ~ 200 nM). Nonspecific binding was measured in the presence of 200 nM unlabeled EGF. The results are displayed as Scatchard plots from the mean of 4 separate competition studies for each group.

pellet was measured in an Aloka ARC-500 Gamma Counter. Specific binding of EGF was calculated by subtracting nonspecific binding measured in the presence of excess unlabeled EGF.

Results. The specific binding of EGF to brain membranes decreased gradually with age in both male and female rats (fig. 1). At 2 weeks of age, the specific binding was 10.2 ± 1.6 fmol and 8.5 ± 1.4 fmol per mg of protein, whereas at 20 weeks of age it was 5.3 ± 0.8 fmol and 4.8 ± 0.6 fmol per mg of protein, in males and females, respectively.

The specific binding was slightly higher in males than in females at 2, 4, 8, 10, and 20 weeks of age; however, there was no significant difference in respective values between the two sexes. Scatchard plots of the binding data for membranes prepared from 2- and 20-week-old rats were almost parallel; and this was true for both male and female rats (fig. 2). The value of the dissociation binding constant (Kd) from each slope was between 6.9 and 7.3 nM. These results indicate that the decrease of EGF binding with age is due to a decrease in the number of receptors.

Discussion. The presence of EGF in various tissues and fluids[10,11] suggests that this peptide may be involved in the control of cellular proliferation and/or cellular function. EGF does not cross the blood-brain barrier[12], and is therefore expected also to be synthesized in the brain. Recently, EGF immunoreactivity has been detected in the rat brain[13]; however, the EGF concentration measured by enzyme immunoassay was shown to be extremely low[14].

The results presented here have demonstrated the presence of EGF receptor in the rat brain, and the receptor concentration was higher in the neonate than in the adult. These results suggest that EGF or EGF-like factor(s) may be involved in the proliferation and differentiation of neural cells in the brain. During the fetal and neonatal periods, the blood-brain barrier is probably incomplete[15]; this raises the possibility that circulatory EGF may also be involved in these functions.

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A pyruvate kinase variant in different mouse transplanted tumors

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Summary. Mouse transplanted tumors, in contrast to normal tissues, contain a pyruvate kinase (PK) variant sensitive to the inhibitory action of L-cysteine and less sensitive to saturated fatty acids than the normal enzyme. In selected normal and tumor materials two fractions of PK were separated. Fraction A (20–30% $(NH_4)_2SO_4$ saturation) dominated in normal liver, and fraction B (50–60% $(NH_4)_2SO_4$ saturation) in skeletal muscles and Ehrlich ascites tumor. Only this fraction B from tumor material was sensitive to L-cysteine, and seems to contain a tumor-specific PK variant which might be considered as a marker of neoplastic transformation in a broad spectrum of mouse experimental tumors.
Key words. Pyruvate kinase; mouse transplanted tumors; tumor marker; L-cysteine; stearic acid.

Pyruvate kinase is one of the key regulatory enzymes of the glycolytic pathway, which is directly involved in cytoplasmatic energy formation [1,2]. Its activity increases significantly during 'spontaneous' or viral cell transformation in vitro [3,4]. In Ehrlich ascites tumor cells this enzyme, in addition to increased activity, acquires a new sensitivity to the inhibitory action of L-cysteine [5–7] and shows a decreased sensitivity to several normal effectors and signal molecules, including saturated fatty acids [8] and ATP [9]. These effects, observed previously both in metabolic studies and in direct enzymatic determinations [5–7] suggested that in tumor cells a PK variant might be present.

The aim of the study now reported was to see whether a PK variant, sensitive to the inhibitory action of L-cysteine and with decreased sensitivity to fatty acids, is specific for a broad spectrum of mouse transplanted tumors, and thus to evaluate its role as a marker of neoplastic transformation.

Material and methods. Cytosolic fractions obtained from various mouse solid and ascites tumors were studied.

Mouse solid tumors (in brackets mouse strains used for transplantations): 1) Mammary adenocarcinoma 16C (C_3H), 2) Madison lung carcinoma (BALB/C), 3) Lewis lung carcinoma (C57BL/6), 4) Colon carcinoma C-28 (C57BL/6), 5) Colon carcinoma C-26 (BALB/C), 6) Melanoma B-16 (C57BL/6), 7) Leukemia L-1210 (DBA-2), 8) Polyoma-induced tumor (DBA-2).

Mouse ascites tumors (in brackets mouse strains used for transplantations): 1) Ehrlich ascites tumor (Swiss), 2) Leukemia L-1210 (DBA 2), 3) Leukemia P-388 (AKR), 4) Leukemia AKSL-4 (AKR).

All solid tumors were transplanted s.c. into syngenic recipients. Ascites neoplastic cells were transplanted i.p. As a rule 10^5–10^6 cells were inoculated by both routes. For comparison cytosolic fractions of normal mouse tissues (liver, skeletal muscle, spleen), and mouse embryo were used.

Tumors and normal tissues obtained from animals sacrificed by cervical dislocation were homogenized with 20 mmol/l Tris-HCl buffer (pH = 7.4), containing 115 mmol/l KCl, 10 mmol/l $MgCl_2$, 1 mmol/l EDTA, in a Potter Elvehjem glass homogenizer, and centrifuged at 100,000 × g at 4 °C for 15 min in a Spinco preparative ultracentrifuge. The cytosolic fractions were used directly for enzymatic studies. In the case of Ehrlich ascites tumor, and for comparison of normal muscle and liver, the cytosol enzyme activity was determined also after ammonium sulphate fractionation between 20 and 70% of saturation, in the range of 10%.

PK activity was determined spectrophotometrically [10] in conditions of pseudozero-order kinetics in the absence or presence of 0.05 mmol/l L-cysteine or 0.05 mmol/l stearic acid in liposomes from 1 mmol/l phosphatidylcholine. The results were expressed in mIU per mg of protein.

Protein concentration was measured by the method of Lowry et al. [11]. The results were analyzed statistically using Student's t-test.

Results and discussion. The activity of PK in cytosolic fractions of various mouse tumors as well as of mouse embryo and normal skeletal muscle was much higher (about 3000–10,000 mIU/mg protein) than in corresponding fractions of mouse spleen or mouse liver (about 250–1000 mIU/mg protein) (table 1).

Stearic acid (0.05 mmol/l) in liposomes decreased cytosolic PK activity in solid tumors (88.8–98.6%) less than in normal tissues studied (59.8–78.9%) when compared with corresponding control activity.

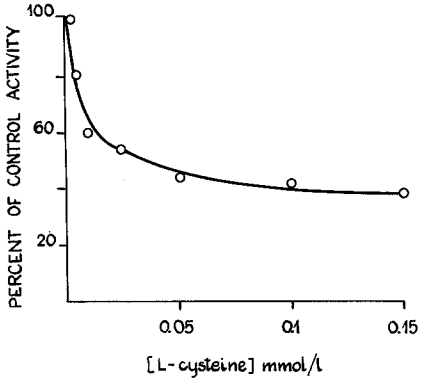

Figure 1. The effect of different concentrations of L-cysteine on PK activity in cytosole of Ehrlich ascites tumor cells. The activity is expressed as a percentage of control value.