cleaning the tissues, a 20% (w/v) homogenate of each tissue was prepared in iso-osmotic sucrose with the help of Potter-Elvehjem homogenizer. The freshly prepared homogenates were used in the experiments. The assay of choline and ethanolamine kinase was as described earlier[7].

Results and discussion. The results obtained for the liver and brain kinase activities in the adult male and female mouse, and in the gravid animal, are given in the table. There was no sex-associated difference in kinase activities, choline kinase and ethanolamine kinase having nearly the same activity in the male and virgin mouse. These findings stood out in contrast to the observation that hepatic serine/threonine dehydratase activity in female rats was $^1/_2$ that in males[8]. Pregnancy induced a differential effect in the phosphorylating activity towards choline and ethanolamine. Ethanolamine kinase was 117% and 80% higher in the liver and brain, respectively, of the pregnant mouse than in the virgin, while the choline kinase activity was unaltered. As a result, the ratio of ethanolamine kinase to choline kinase was almost doubled in the pregnant animal. Surprisingly there were no sex-associated differences and gestation-induced changes in choline and ethanolamine phosphorylating activities in the male, virgin female and gravid mouse kidney tissues. Solyom and Lauter[9] found that the specific activity and enrichment of several hepatic plasma membrane marker enzymes were significantly lower in the female than male rats. The lipid composition showed no comparable sex differences. In brain membrane fractions, similar sex difference in enzymatic activities did not exist. Pregnancy is attended by changes in the activities of a number of enzymes. These are not confined to the placenta and the mammary gland but extend to other tissues. Diamant and Shafrir[10] found gestation-induced changes in the activities of a number of hepatic enzymes of carbohydrate and lipid metabolism. The present studies suggest that ethanolamine kinase, but not choline kinase, can be induced by the action of female sex hormones. Further work regarding the effect of steroid hormones etc. is under progress. The increased ethanolamine kinase activity in the pregnant animal may be due to increased rate of synthesis of the enzyme or to a decreased rate of degradation or both.

The increase in activity under the stress of pregnancy suggested that phosphorylation of ethanolamine was a rate-limiting action. Assuming that the relative content of choline- and ethanolamine phospholipids did not alter in pregnancy, it would appear that ethanolamine phospholipids were biosynthesized de novo, and choline phospholipids derived therefrom by transmethylation. The preferential increase which occurred in ethanolamine phosphorylating activity in the pregnant animal also suggested, as well as lent support to the previous findings[7,11] that different proteins catalyzed the phosphorylation of choline and ethanolamine, or that a common enzyme catalyzed the phosphorylation of the 2 bases in the virgin, but that a specific enzyme catalyzing the phosphorylation of ethanolamine was induced under the physiological stress of pregnancy. It has long been a matter of controversy whether 'ethanolamine kinase' is a separate enzyme or choline kinase acts on both choline as well as ethanolamine. Now it seems quite convincing from previous[7,11] and present findings that 'ethanolamine kinase' is a separate enzyme, and it can be listed in the official list as an enzyme of its own.

Choline and ethanolamine kinase activities in liver and brain of gravid- and virgin mice

		Choline kinase units/mg protein	Ethanolamine kinase	Ratio (ethanolamine kinase/choline kinase)
Male	Liver	0.032±0.001	0.54±0.04	16.5
	Brain	0.043±0.004	0.81±0.06	18.8
Virgin	Liver	0.032±0.001	0.60±0.03	18.7
	Brain	0.041±0.003	0.86±0.08	21.0
Gravid	Liver	0.035±0.001	1.29±0.04	38.0
	Brain	0.044±0.004	1.54±0.08	35.0

All animals were about 4-month-old. The gravid animals had conceived for the first time. The values reported are the mean of 8 estimations with SD.

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Anomeric compositions of D-glucose in tissues and blood of rat

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Summary. The anomeric compositions of D-glucose in the liver, kidney, heart, blood and plasma of rat were determined by our method for the assay of D-glucose anomers and the percentages of the β-anomer were found to be 61.8, 61.0, 62.4, 62.7 and 62.9, respectively.

D-Glucose is known to exist as an equilibrium mixture of its 2 anomers in aqueous solution; 36% α-D-glucose and 64% β-D-glucose[2,3]. Almost the same anomeric composition is also found in blood[4]. The percentages of the α- and β-anomers of intracellular D-glucose, however, could not yet be determined, mainly due to technical difficulties, inspite of its necessity for the study of the physiological function of D-glucose anomers.

This paper describes the method for determining the anomeric compositions of D-glucose in tissues and blood samples, and the results obtained on the liver, kidney, heart, brain, adipose tissue, blood and plasma of rat.

Materials and methods. Male Wistar strain rats weighing 200–250 g were given free access to standard rat chow and tap water. Rats fasted for 24 h before use were decapitated to bleed out of tissues as much as possible. Blood

was collected in a test-tube moistened with heparin solution, cooled in ice and separated to plasma and erythrocytes fractions by centrifugation at 4 °C. The liver, kidney, heart, brain and adipose tissue were quickly removed off, and the tissues and blood samples were immediately frozen in dry ice-acetone. It took only 80 sec to finish the removal of tissues after decapitation. Frozen tissues (0.7–1.5 g) and blood samples (around 1 ml) were homogenized in 20 volumes of an ice-cold mixture of chloroform and methanol (10:1, v/v) supplemented with 10 µl of 0.2 M raffinose (see below for the reason of addition) with a Polytron homogenizer (Kinematica, Luzern, Switzerland). The homogenates were centrifuged at 10,000 × g and 4 °C for 5 min. The upper layer (aqueous layer) thus obtained served as a sample solution for the assay of D-glucose anomers, because D-glucose in the lower layer (chloroform layer) was only less than 2% of the total D-glucose when determined with D-[^{14}C(U)]-glucose (New England Nuclear, Boston, USA) as a tracer, i.e. D-glucose in tissues and blood samples was almost entirely extracted in the aqueous layer. The assay of D-glucose anomers in sample solutions was carried out by our method[3,5] using β-D-glucose oxidase (β-D-glucose:oxygen oxidoreductase, EC 1.1.3.4), mutarotase (aldose 1-epimerase, EC 5.1.3.3), and an oxygen electrode within 30 min after the preparation of sample solutions. In the present assay, a sample solution was added to the buffer solution in a vial prior to the addition of β-D-glucose oxidase. The principle of this assay was that β-D-glucose in the sample was oxidized with the consumption of oxygen (referred as A), α-D-glucose remaining in the solution was converted to β-D-glucose by addition of mutarotase with subsequent consumption of oxygen (referred as B) and consequently the percentages of the α and β anomers of D-glucose can be determined as 100B/A + B and 100A/A + B, respectively. The volume of the sample solution was 10 µl for liver and 50 µl for kidney, heart, brain, adipose tissue, blood, and plasma. The total volume of the aqueous layer which is needed for the calculation of D-glucose amount in tissues and blood was determined by the dilution analysis using raffinose as a water-soluble tracer. Raffinose was assayed by the method of Roe et al.[6].

Results and discussion. The difficulty in assaying D-glucose anomers in tissues and cells was thought to be mainly due to the rapid mutarotation of D-glucose in homogenates, to the dilution of cell fluid (containing D-glucose) on homogenization and to the presence of D-glucose-forming and D-glucose-consuming enzymes. In order to overcome these problems, we devised a unique treatment of tissues which consisted of rapid freezing of tissues followed by homogenization in a mixture of chloroform and methanol. The usage of 20 volumes (per g wet wt) of a mixture consisting of 10 volumes of chloroform and 1 volume of methanol was chosen as the best method. It was confirmed that the following interfering-proteins and -enzymes were denatured by this treatment; mutarotase (accelerate the mutarotation of D-glucose anomers), catalase (decompose hydrogen peroxide to oxygen and water), hemoglobin (bind with oxygen), hexokinase and glucose-6-phosphatase. The mutarotational rate of D-glucose was found to be considerably slower in a sample solution which was obtained from the homogenate of kidney (1.2 g) supplemented with 20 µl of 50 mg/ml α-D-glucose, i.e. the percentages of β-D-glucose in the sample solution just after its preparation and at 1 h after that were 51.2 and 52.1, respectively. Another advantage of this treatment was that D-glucose in tissues was concentrated in small quantities of the aqueous layer, i.e. the volume of the aqueous layer was 0.65–0.75 ml for liver, 0.86–0.95 ml for heart, 0.98–1.07 ml for kidney, 0.82–0.93 ml for blood and 1.30–1.43 ml for plasma when 1 g of each tissue or 1 ml of blood and plasma was used.

The anomeric compositions and total amounts of D-glucose in tissues and blood samples are summarized in the table. These anomeric compositions were near to mutarotational equilibrium, but had a significant tendency to be abundant in the α anomer. Since both whole blood and its plasma showed almost the same anomeric compositions, D-glucose anomers in erythrocytes would also occur nearly at those compositions. Statistical analysis was performed by means of the Student's t-test. Since some volume of blood is contained in tissues inspite of the bleeding caused by decapitation, blood D-glucose forms a part of D-glucose found in tissues. Blood D-glucose, however, was supposed to be less than 5% of total D-glucose extracted from tissues. Therefore, the values of the anomeric compositions of D-glucose in tissues listed in the table would be roughly equivalent to that in intracellular fluid of tissues.

It was reported that β-D-glucose was more rapidly transported than α-D-glucose into various cells and tissues studied hitherto, e.g. human erythrocytes[7], Ehrlich ascites tumor cells[8], rat pancreatic islets[9], rat retina[10], and rat brain slices[11], so the percentages of β-D-glucose in tissues and erythrocytes were expected to be larger than that in equilibrium D-glucose. The present findings, contrary to anticipation, could be due to the intracellular preferential phosphorylation of β-D-glucose by hexokinase which was suggested by our preliminary experiment.

Anomeric compositions and total amounts of D-glucose in rat tissues and blood samples

Tissue and blood sample	No. of experiment	α-D-Glucose[a] (%)	β-D-Glucose[a] (%)	Total D-glucose (µg/g wet wt)
Liver	7	38.2±0.53	61.8±0.53[b]	1490±571
Kidney	7	39.0±0.83	61.0±0.83[b]	337± 56
Heart	7	37.6±0.35	62.4±0.35[b]	401± 70
Brain	3	NA	NA	Trace
Adipose tissue	3	NA	NA	Trace
Whole blood	5	37.3±0.74	62.7±0.74[c]	64± 3[d]
Plasma	5	37.1±0.57	62.9±0.57[c]	67± 4[d]

The percentages of the α- and β-anomers of equilibrium D-glucose were 35.6 ± 0.36 and 64.4 ± 0.36 (mean ± SD, 7 determinations), respectively. [a]Mean ± SD; [b]$p < 0.001$, [c]$p < 0.01$ when compared to the values of equilibrium D-glucose; [d]mg/dl; NA, not analyzed.

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The anomeric compositions of D-glucose in sample solutions became almost the same as the values (35.6% α, 64.4% β) of equilibrium D-glucose in all cases of tissues, and blood samples after sample solutions were kept for 24 h at room temperature (20 °C) to cause D-glucose to mutarotate completely. This result supports the accuracy of the percentage values in the table obtained on fresh sample solutions.

Our colorimetric method[12] for determining D-glucose anomers was tested instead of the present oxygen electrode method, but it was not utilizable for this study because of the interference of color development by reducing substances contained in sample solutions.

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Effect of pentobarbital on the synaptosomal activity of acetylcholinesterase in Mongolian gerbils

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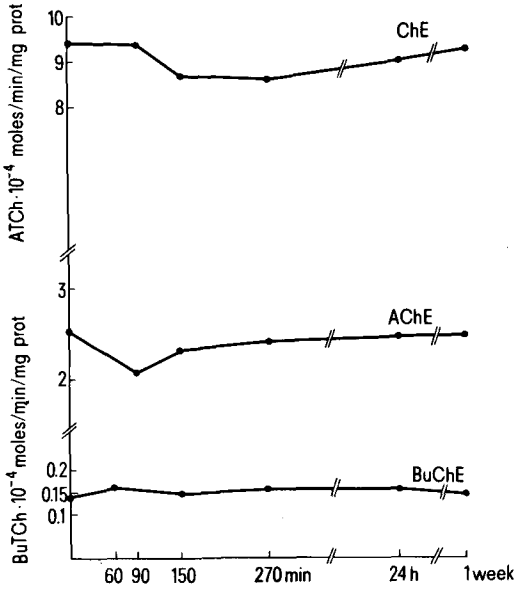
Summary. The influence of Na pentobarbital anesthesia on the activity of specific and nonspecific cholinesterase was studied in the synaptosomal fraction of Mongolian gerbils' brains. These studies have shown that this barbiturate inhibits the specific activity of acetylcholinesterase only.

Barbiturate reduction of either specific or nonspecific cholinesterase was not observed in early postanesthetic period although high concentrations of phenobarbital were described to inhibit in vitro the hydrolysis of acetylcholine in rat brain[1] and in vivo studies to raise the acetylcholine level in rats and cats[2]. In this communication we will describe a transient effect of pentobarbital on the activity of acetylcholinesterase in synaptosomal fraction of Mongolian gerbil's brain.

The gerbils (50–75 g) were anesthetized by i.p. Na pentobarbital injection (20 mg/kg b.wt) and killed at various intervals from 90 min to 1 week following the treatment. Each group consisted of 6–12 experimental and control animals. After decapitation the brains were removed quickly and transferred immediately in 0.32 M sucrose at pH 7.0. The synaptosomal fraction was prepared according to the method of Whittaker and Barker[3].

Acetylcholinesterase (AchE EC 3.1 1.7) activity was assayed in 100 μl aliquots of the fraction by spectrophotometric method of Ellman et al. using acetylcholine as substrate and tetraisopropyl-pyrophosphoramide for inhibition of pseudocholinesterase[4]. Butyrylcholinesterase activity was assayed by the same method but using butyrylcholine (BuTCH) as substrate.

The acetylcholinesterase was the only enzyme affected by the pentobarbital anesthesia. The activity of this enzyme was 27% lower in the experimental than in control brains 90 min after treatment. The reduction was transient since the acetylcholinesterase activity returned to almost normal levels at 150 min. No significant changes of cholinesterase or butyrylcholinesterase activity were seen between the anesthetized and control animals (see figure and table). These findings are different from the ones described by Vernadakis, who observed a markedly



The points represent mean values of 6–12 experiments described in the text. The exact numbers with MEM are illustrated in the table.

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Specific and nonspecific cholinesterase activity

Projection Time	AChE	ChE	BuChE
Control	2.518 ± 0.050++	9.428 ± 0.475	0.141 ± 0.009
90 min	2.090 ± 0.083*	9.432 ± 0.396	0.159 ± 0.006
150 min	2.356 ± 0.080	8.745 ± 0.350	0.144 ± 0.014
270 min	2.398 ± 0.076	8.673 ± 0.180	0.157 ± 0.016
24 h	2.455 ± 0.012	9.150 ± 0.270	0.156 ± 9.011
7 days	2.493 ± 0.050	9.316 ± 0.056	0.145 ± 0.0093

*$p < 0.01$; ++means ± SE of 10^{-4} moles substrate hydrol./min/mg protein.