with L-glutamine as substrate assayed under the same conditions. However, the apparent K_m for glutamine as measured by the respiration rate was several times lower than for glutamate (1.4 mM and 16.6 mM for glutamine and glutamate respectively). To investigate whether glutamine oxidation is taking place through glutaminase pathway (L-glutamine amidohydrolase EC 3.5.1.2.) but not through transamination with keto acids catalyzed by glutamine transaminase (L-glutamine: 2 oxoacid aminotransferase, EC 2.6.1.15), the formation of the reaction products was determined. The figure shows the dependence on time of ammonia and glutamate formed in the presence of an inhibitor of respiratory chain. It appears that the first step of glutamine oxidation by rat skeletal muscle mitochondria is the hydrolysis of glutamine by glutaminase (L-glutamine amidohydrolase EC 3.5.1.2). Ammonia production from glutamate was very low under these conditions: at the end of the experimental period, less than 0.2 μmoles from glutamate was formed. Inhibition of glutamine and glutamate oxidation by malonate and amino-oxyacetate (not shown) suggests that these amino acids are oxidized mainly through the transamination pathway similarly as in mitochondria from other tissues[16–19]. Glutamate added or formed by hydrolysis of glutamine would undergo transamination with oxaloacetate, to form aspartate and 2-oxoglutarate. The latter would be oxidized through the Krebs cycle supplying further oxaloacetate for transamination. Inhibition by malonate of glutamine and glutamate oxidation could have been completely reversed by a low concentration of either L-malate or fumarate (not shown). These results also indicate that these amino acids are oxidized mainly through the transamination pathway. In this case oxaloacetate may be formed only from the malate or fumarate added, as malonate prevents the conversion of 2-oxoglutarate to oxaloacetate by inhibiting succinate dehydrogenase.

The results obtained in this study indicate that isolated rat skeletal muscle mitochondria are able to oxidize glutamine and that the first step of this process is deamination of glutamine via the pathway catalysed by glutaminase. It seem reasonable to postulate that in the muscle of the exercising rat, glutamine may be oxidized as one of the energy sources. The observation that in the exercising rat a significant decrease in plasma and tissues glutamine concentration occurs[6], may be pertinent in this respect.

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Search for sex-dependent and gestation-induced changes in choline and ethanolamine phosphorylating activities

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Summary. Choline kinase and ethanolamine kinase of liver, brain and kidney had nearly the same activity in 4-month-old male and virgin female mice. Ethanolamine kinase activity was almost doubled in the liver and brain of mice in advanced pregnancy compared with the virgin, while choline kinase activity was unaltered.

Sex-linked differences in enzyme activities and sex-specific enzyme activities and pregnancy-induced changes in enzyme activities have been documented[2]. Gulonolactonase in adult rat kidney, but not liver, exhibits sex dependence in activity and is controlled by androgens. The renal level of the enzyme in the male was 10–15-fold that in the female[2]. The activities of enzymes involved in the metabolism of steroid hormones in rat liver exhibit distinct sexual differences[3–5]. There is a striking sex-difference in the activity of liver histidine ammonialyase which was ascribed to induction of the enzyme forming system by oestrogen during pubertal development[6].

Several of the enzymes showing sex-dependent changes in activity are membrane-associated. Phospholipids are known to influence the activity of a number of membrane-bound enzymes, and in many of these cases the requirement is for a specific phospholipid. It was, therefore, of interest to examine whether sex-dependent change existed in choline- and ethanolamine kinase activity in liver, brain and kidney tissues, which are the active tissues involved in the phospholipid synthesis. During the gestation period there is additional requirement for phospholipids, and it is possible that there are differences in the requirement for choline phospholipids and ethanolamine phospholipids. The following study is a search for a possible sex-dependence in the phosphorylative activity towards choline and ethanolamine, and for any changes in the activity in the pregnant animal. A difference in the ratio of ethanolamine kinase to choline kinase between the male and the female adult, and between the virgin and gravid female, would be indicative of separate proteins catalyzing the phosphorylation of choline and ethanolamine.

Materials and methods. The mice used were 4-month-old. The 17–20 days pregnant animals used in the study had conceived for the 1st time. The animals were housed under standard conditions and fed the standard colony diet. The animals were sacrificed by cervical dislocation and decapitation. The liver, brain and the pair of kidneys were excised rapidly and kept chilled. The gall bladder from the liver was cut and discarded. After chilling and

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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