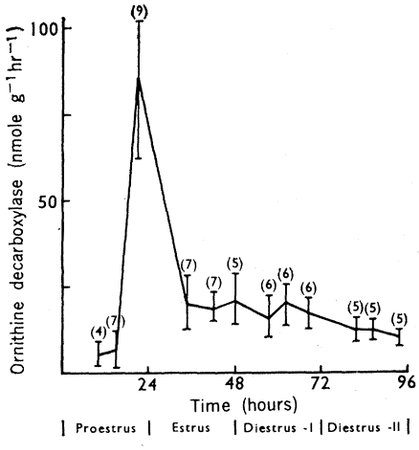

Fig. 1. Ornithine decarboxylase in the rat ovary during the estrous cycle. Rats with a 4-day cycle were used, and animals were killed at 10 a.m., 2 p.m., and 8 p.m. The number of animals in each group is indicated in parentheses and enzyme activity is expressed as nanomoles (±S.E.) of CO_2 per gram of tissue per hour.

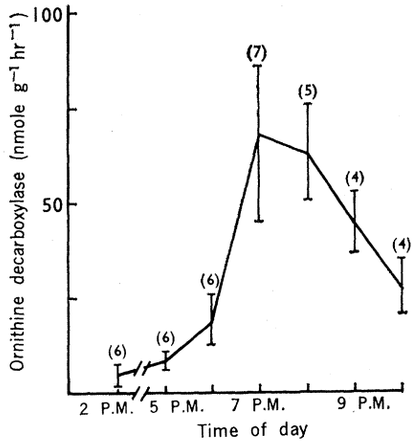

Fig. 2. Ornithine decarboxylase in the rat ovary during late proestrus. Rats with a 4-day cycle were killed, as indicated, during late proestrus between 2 p.m. and 10 p.m. The number of animals in each group is indicated in parentheses, and enzyme activity is expressed as nanomoles (± S.E.) of CO_2 per gram of tissue per hour.

izing hormone (LH) release occurs on the afternoon of proestrus, usually between 3 and 7 p.m., and ovulation occurs about 12 hours later (*7*). Therefore, LH and a variety of other hormones were tested as follows: rats in proestrus were injected subcutaneously at 9 a.m. with the designated hormone, and the ovarian ornithine decarboxylase activity was determined 4 hours later (Table 1). Both LH and human chorionic gonadotrophin (HCG) produced a substantial increase in enzyme activity, whereas the other hormones had little or no stimulatory effect upon this enzyme. In fact, thyroxine appeared to be inhibitory. The results with HCG and follicle-stimulating hormone (FSH) may have been due to the presence of LH, which is known to contaminate these two hormones. In order to test this hypothesis further, rats at proestrus were injected at 2 p.m. with bovine antiserum to LH, which can neutralize rat LH, and the ovarian ornithine decarboxylase activity was measured at 7 p.m. and compared to that of untreated rats (Table 2). The antibody to LH prevented the rise in enzyme activity which normally occurs on the evening of proestrus; these results suggest that ovarian ornithine decarboxylase activity is under LH control.

In the rat, there are now four hormones that induce this enzyme in the appropriate target tissue: testosterone in the prostate (*5*), estradiol in the uterus (*8*), growth hormone in the normal and regenerating liver (*1–3*), and LH in the ovary. The short half-life (10 to 15 minutes) of induced ornithine decarboxylase in regenerating liver (*9*), the hormone specificity of tissue response, and the rapid inducibility of the enzyme suggest to us that polyamine biosynthesis is a finely modulated process closely associated with the metabolic activity of growing tissues that are under hormonal regulation. In the rat ovary, the strategic positioning of the rise in ornithine decarboxylase activity between the release of LH and ovulation implies that polyamines may be intimately associated with the ovulatory process and may be related to the synthesis of new protein and RNA believed to be involved in the early phase of LH action (*10*). However, whether specific inhibition of ornithine decarboxylase would influence ovulation is not known. Nonetheless, the possibility that the control of ovulation might be achieved at a site more specific than that afforded by the pituitary-ovarian axis should stimulate the search for an inhibitor of ornithine decarboxylase.

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Acetoacetyl–Coenzyme A Thiolase in Brain, Liver, and Kidney during Maturation of the Rat

Abstract. *The development of thiolase in various rat tissues from 7 days before birth to adulthood was studied. Enzyme activities in brain, liver, and kidney during the perinatal period reflect the nutritional environment, whereas those in the adult tissues do not.*

Itoh and Quastel (*1*) have shown that in slices of immature rat brain acetoacetate was a more potent source of acetyl-coenzyme A (CoA) than glucose. The sequential pathway of acetoacetate degradation involves acetoacetyl-CoA transferase (E.C. 2.8.3.5) and acetoacetyl-CoA thiolase (E.C. 2.3.1.16) neither of which was measured by Itoh and Quastel. Thiolase furthermore is implicated in the beta oxidation of fatty acids. Inasmuch as the young rats during the first 3 weeks of life are nursing and thus are getting a diet high in fat, it became important to ascertain whether thiolase activity of brain and possibly of kidney reflects the increased utilization of ketone bodies and furthermore whether thiolase activity of liver would be an index of increased fatty acid degradation and ketone body formation. It was for these reasons that the development of thiolase activity was followed in rat brain, liver, and kidney during maturation of these tissues.

All experiments were conducted with albino Wistar rats. No sexual differentiation was made of rats less than 4

weeks of age; adult animals, however, were always males. Rats were killed by decapitation after light ether anesthesia; pups and young rats were killed without previous anesthesia. Brain, liver, and kidney were quickly excised and chilled. From pregnant rats only placenta, fetal brain, and fetal liver were used. The tissues were made into 10 percent homogenates with ice-cold 0.25*M* sucrose containing 0.3 percent deoxycholate (DOC) unless otherwise stated and were centrifuged for 15 minutes at 12,000*g* in a refrigerated centrifuge (International). Thiolase was determined in the supernatant as described by Williamson *et al.* (*2*). The method is based upon measurements of the disappearance of acetoacetyl-CoA at 313 nm. The millimolar absorption coefficient for acetoacetyl-CoA was found to be 11.9 which is in agreement with the value of 11.8 reported by Williamson *et al.* (*2*). Protein was determined by the Lowry method (*3*). Thiolase activity was expressed as nanomoles of acetoacetyl-CoA converted per milligram of protein per minute.

Thiolase in rat liver is mostly particulate (*2*) and requires a solubilizing process such as ultrasound (*2*) or freeze-thawing (*4*) to liberate total enzyme activity. Either technique is more time-consuming than the single-step operation of adding a detergent such as DOC to the homogenizing medium. Table 1 demonstrates that DOC effectively solubilized latent thiolase activity in liver and kidney, as compared to freeze-thawing of water homogenates. As the detergent neither increased nor decreased the enzyme activity in brain and placenta, it was used routinely in the preparation of all tissue homogenates.

Thiolase activity could be measured in fetal tissues several days before birth (Fig. 1). At day 15 of gestation fetal brain, fetal liver, and placenta had equal amounts of thiolase activity. At this point the development curves for thiolase diverged. The placental enzyme decreased progressively with gestation. The brain enzyme showed a slight increase around birth, but otherwise remained fairly constant for the first 3 weeks of life. The thiolase activity in adult brain was less than one-half of that found at birth. In liver and to a lesser extent in kidney the enzyme increased rapidly around birth, and peaks were noted at 16 days for the liver and at 20 days for the kidney. A comparison of enzyme activities at birth and in the adult showed that those of liver were equal and that those of kidney were significantly ($P < .01$) higher in adulthood than at birth. Thiolase activities of adult liver and kidney were not significantly different from each other, but each was five times greater than that of brain.

Table 1. Effect of deoxycholate (DOC) on thiolase activity of adult rat tissues. Ten percent homogenates were prepared in 0.25*M* sucrose, in 0.25*M* sucrose containing 0.3 percent DOC, or in water. The latter were frozen and thawed twice. All homogenates were centrifuged for 15 minutes at 12,000*g* in the cold. Thiolase activity in the supernatant is expressed as the mean ± 1 standard deviation of the number of nanomoles of acetoacetyl-CoA removed per milligram of protein per minute. The number of animals used is shown in parentheses.

Tissue	H_2O	DOC	
		Omitted	Added
Brain		13.5 ± 2.8 (3)	13.8 ± 2.8 (8)
Liver	67.3 (2)	30.8 ± 4.5 (6) *	75.6 ± 13.3 (8) †
Kidney	57.0 (2)	32.5 ± 2.6 (3) *	61.3 ± 10.5 (6) †
Placenta ‡		15.5 ± 1.0 (3)	15.6 ± 3.5 (3)

* Difference between adjacent values significant at $P < .01$. † Not significantly different at $P < .01$. ‡ Six placentas were pooled from each 18-day gestational animal.

Table 2. Effect of starvation on thiolase activity (nanomoles per milligram of protein per minute) of brain and liver. Adult male rats had their food withdrawn and were given water only for the periods stated. At decapitation, blood was collected and total ketone bodies (*10*) and cholesterol (*11*) (milligrams per 100 ml) were determined in the serum. Brain and liver were homogenized in DOC-sucrose and centrifuged. Thiolase was assayed in the supernatant. Results are shown as the means ± 1 standard deviation.

Starvation (hr)	Rats (No.)	Thiolase activity (nmole mg^{-1} min^{-1})		Serum ketones (mg/100 ml)	Serum cholesterol (mg/100 ml)
		Brain	Liver		
None	8	13.5 ± 2.8	75.6 ± 13.3	3.0 ± 0.7	85 ± 5
24	4	19.0 ± 2.5 *	77.3 ± 10.8	10.4 ± 2.8	111 ± 9
48	6	15.5 ± 3.0	147.6 ± 23.0 †	8.8 ± 2.2	110 ± 3
72	5	16.0 ± 1.1	82.0 ± 10.5	10.5 ± 2.4	128 ± 3

* Not significantly different ($P < .01$) from controls. † Significantly different ($P < .01$) from controls.

The results are compatible with the functional characteristics of these organs. Data obtained during the prenatal period are indicative of placental steroid synthesis, in which thiolase is a key enzyme, and correlate well with the placental uptake and transfer of free

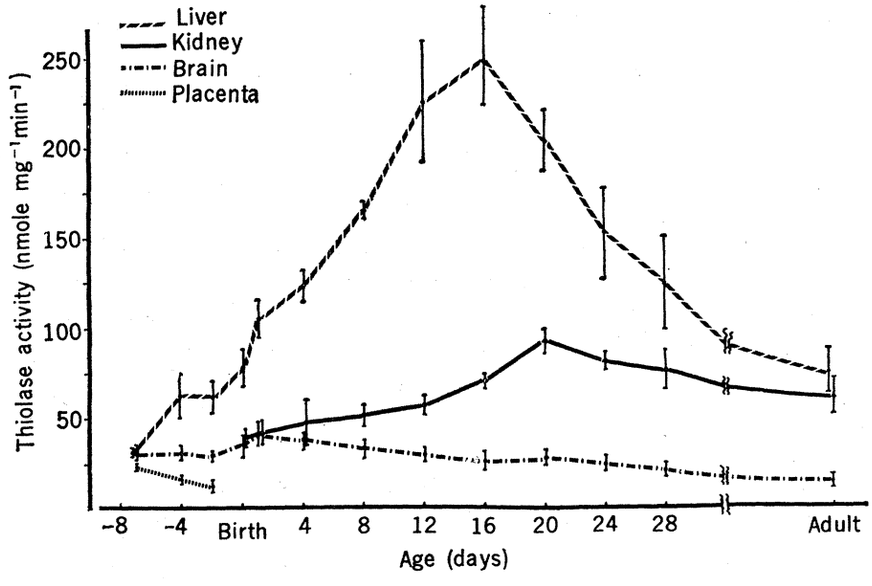

Fig. 1. Development of thiolase activity in rat tissues. Data before birth were obtained with timed-pregnancy rats (vaginal smear method). For each point on the curves, three pregnant rats were used; from each one, six placentas and the same number of fetal brains and livers were pooled. Pregnant rats with less than eight fetuses were discarded. After birth, six pups from at least two different litters were used for each point of the curves. Individual tissues were used throughout except at birth where tissues from three rats were pooled, and 18 rats were used in total. Each point is the mean ± 1 standard deviation.

fatty acids (*5*) and of ketone bodies (*6*). However, ours is the first demonstration of the existence of thiolase in prenatal tissues.

After birth, the development of thiolase activity reflects the nutritional environment of the young rats whose nourishment for the better part of the first 3 weeks of life is maternal milk, which is high in fats. Evidence for the expected increased breakdown of fatty acids and ketogenesis by the liver was provided by its thiolase activity which increased threefold between birth and weaning. During the same period, brain thiolase activity was maximum. The fact that the kidney can utilize as well as produce ketone bodies is reflected in a pattern of thiolase development intermediate between that of liver and brain.

Another and similar index of the nutritional environment of the growing rats is D-(−)-β-hydroxybutyrate dehydrogenase which has been shown by Klee and Sokoloff (*7*) to rise immediately after birth in rat brain, to reach a maximum at age 30 days, and to fall to low levels in adulthood. This enzyme could then be reinduced by starvation (*8*) or by a diet high in fat (*9*). To test whether thiolase would exhibit a similar behavior, we subjected adult rats to starvation for periods up to 72 hours and determined the thiolase activity in brain and liver (Table 2). Although the rats became ketonemic after 24 hours and also showed increased cholesterol in the serum, brain thiolase activity was not elevated significantly and the increase of liver thiolase activity seen temporarily at 48 hours of starvation could not be correlated with concentrations of ketone bodies or cholesterol in the serum. Thus it seems that there is no need for adult brain thiolase to adapt during fasting.

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Immunoglobulin Production by Human Lymphocytoid Lines and Clones: Absence of Genic Exclusion

Abstract. *Immunoglobulin production was studied in established lines of normal human lymphocytes. Three lines which produced both immunoglobulin G and immunoglobulin M were cloned. Among the 25 immunoglobulin-producing clones, 23 produced both classes of immunoglobulins. These findings suggest that the phenomenon of genic exclusion does not hold for immunoglobulin production in lymphocytoid cells in culture.*

The development of techniques for establishing human lymphocytes in long-term culture (*1*) and more recently for cloning these cells (*2*) has enabled us to test the widely held view that single cells are capable of producing only a single, heavy chain class of immunoglobulins (*3*). On the basis of immunodiffusion studies of the concentrated medium in which the lymphocytes grow, it appears that clonal sublines, established from parental lines that produce both immunoglobulin G (IgG) and immunoglobulin M (IgM) of the kappa type, actively synthesize both classes of immunoglobulins, of the same type specificity as the parental lines.

Peripheral blood lymphocytes from three hematologically normal persons were established with the use of lysates of previously established lymphocytoid lines, as described by Choi and Bloom (*1*). These three lines, designated UM-1, -5, and -21 (University of Michigan, first, fifth, and twenty-first lymphocyte lines, respectively), were established in the course of our cytological, biochemical, and immunological studies on lymphocytes in long-term culture. All had been established and subcultured for at least 3 months prior to the cloning experiments; and all were, on karyotype analysis, normal diploid cell lines. Occasional aneuploid and tetraploid cells were seen, but these never exceeded 15 percent of the examined cells of each line.

In order to test the hypothesis of genic exclusion for the immunoglobulin loci, a number of clonal sublines was established from each of the three parental lines. The cloning technique has been described in detail elsewhere (*2*), with approximately 50 percent of isolated, single cells, from established lymphocytoid lines, going on to form clones. Single cells from the lines were isolated in individual wells of plastic serotest plates (Falcon) and incubated for 4 to 8 weeks, with media changes and transfer to larger culture vessels, until clonal sublines were established. Eleven clones were established from the UM-1 line, eight from the UM-5 line, and ten from the UM-21 line.

When the concentration of cells in the cultures of the parental and clonal lines reached 1×10^6 cells per milliliter, medium was obtained for immunoglobulin studies. Medium was obtained by decanting after centrifugation of the cells and medium at 1500 rev/min for 10 minutes. The cells were then returned to culture with fresh medium added. Since no effort was made to synchronize the cells, they were in various stages of interphase and mitosis at the time the medium was tested for immunoglobulin production (*4*). The used medium was tested for the presence of immunoglobulins, after a 40-fold concentration of it had been obtained by vacuum suction. Concentrates were stored at −4°C until tested.

Immunoglobulins were studied by a modification (*5*) of the double diffusion method of Ouchterlony (*6*), by using Oxoid Ionagar No. 2 (Colab), with a barbital buffer of *p*H 8.5. Equine antiserums (Kallestad) to human IgG (γ chain specific) and IgM (μ chain specific), and to kappa and lambda light chains, were placed in the center wells of the agar-covered slides, and run against the concentrated medium placed in the peripheral