ratio, U^{234}/U^{238}. If V_1 is unity, the following equations are derived:

$$M_2 = C_1 (A_1 - A_3)/(A_3 - A_2) \quad (5)$$

$$V_2 = [(C_1 + M_2)/C_3] - 1 \quad (6)$$

These expressions can be used in two ways: (i) in calculating the relative mixing volumes of two waters when C and A values for the two source waters (1 and 2) and the resultant mixed water (3) have been determined, and (ii) in deducing the amount of uranium and water (2) that have been added to an initial water (1) to produce a resultant water (3). In the latter case, there are three unknowns, M_2, A_2, and V_2, in Eqs. 5 and 6; one of these variables must be estimated.

For example, we might ask: what volume of water such as that from Horn Spring (2) must be mixed with a unit volume of water such as that from Big Bend (1) to produce water such as that flowing from Wakulla Spring (3) (Fig. 1)? From Eqs. 5 and 6, M_2 is 8.4, and V_2 is 17.4. Thus, for every liter of Big Bend water, 17.4 liters of Horn Spring water would be required to yield 18.4 liters of Wakulla Spring water. As a check on the uranium balance, we can compute the necessary concentration of Horn Spring water ($C_2 = 0.48$ μg/liter), which is almost precisely the value measured.

There are probably numerous hydrologic situations in which such "closed system" assumptions can be made, the C and A values of three waters determined, and the mixing volumes calculated with confidence and accuracy. Requirements include sufficient uranium (0.1 μg/liter or more), diverse activity ratios, and a reasonable understanding of the hydrologic system. Isotope dilution analysis cannot define a mixing model; it does, however, set limits on possible models and develops the implications of these.

As an example of the second approach, we refer to Fig. 1, in which successive aquifer points are compared, and the additions of uranium and water between points are deduced. This approach is useful because the aquifer system here is quite open; rainwater is infiltrating from the surface, and uranium is being leached from the aquifer rock. In this case the observed variations in the activity ratios of waters within the aquifer are systematic enough to permit most probable values for A_2 to be assigned (*12*) and values for M_2 and V_2 to be calculated.

The results of these calculations of successive intervals are shown in Table 1, in which the relative volumes, as well as the amounts of uranium which are added between Havana (H), Tallahassee (T), Big Bend (B), and Wakulla Spring (W) are computed. (The larger interval, Tallahassee to Wakulla Spring, is also computed for comparison.) Since each V_2 is defined relative to each initial V_1, the total volume increment to the final water can be determined (last column of Table 1). Our calculations show that, of the hypothesized sources for Wakulla Spring, the more local sources predominate, and that no more than about 8 percent could be contributed from as far away as Havana.

Although these results must be regarded as semiquantitative only, because of the openness of the hydrologic system, they support the model and provide limits for mixing proportions. The activity ratios used may also be anomalously low; nevertheless, we believe that this analysis illustrates the general applicability of studies of uranium isotopes to hydrologic investigations.

It is unlikely that any other element will be useful in just this way. Thorium (Th^{232}, Th^{230}) has a greater range of activity ratios in water, but it occurs in very low concentrations. Hydrogen, carbon, and radium also exhibit measurable isotopic variations; however, their relatively short half-lives, which make them of interest as a possible means of absolute age-dating of water, limit their usefulness as indicators of mixing proportions.

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12. In estimating the A_2 value (activity ratio) of the uranium leached between sampling points 1 and 3, the following aspects were considered: (i) aquifer waters here show a generally decreasing activity ratio for their dissolved uranium in the direction down the slope of the piezometric surface; (ii) surface waters generally have activity ratios greater than 1.00, in agreement with the theory of isotope fractionation by leaching; (iii) $A_2 - A_3$ must have the same sign as $A_3 - A_1$; (iv) whenever C_3 is significantly greater than C_1, $A_2 - A_3$ must be small. As a result of these considerations, all A_2 values were selected such that the range of $A_2 - A_3$ was 0.02 to 0.10, except for the portion from Tallahassee to Big Bend where it was 0.01 to 0.06. Infiltrating waters were assumed to contribute only small amounts of uranium; the activity ratio of these waters is 0.90+ (Fig. 1).
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Tyrosine Transaminase: Development of Daily Rhythm in Liver of Neonatal Rat

Abstract. *The development of the 24-hour rhythm in tyrosine transaminase activity was studied in the liver of the neonatal rat. A recognizable rhythm appears within 48 hours after birth, but it is opposite in phase to that observed in the adult rat. The reversal of the adult pattern occurs 21 to 23 days after birth and may represent a response to a change both in the eating pattern and in the amount of protein eaten.*

A daily rhythm in the activity of the liver enzyme L-tyrosine-2-oxoglutarate aminotransferase (TT) (E.C.2.6.1.5.), which is generated by intake of a diet (*1*), occurs in the adult rat (*2*). We have studied the development of this cycle in the neonatal rat.

Female CFE rats (Carworth) were obtained during the second week of pregnancy. During gestation and at all times thereafter, the animals were kept in large litter cages maintained with controlled temperature and humidity. Rats were given free access to an 18-

percent casein agar-gel diet. The lights were kept on from 6 a.m. to 6 p.m. each day. At indicated intervals after birth, pups were selected at random from the litters and decapitated at 10 a.m. or 10 p.m. Livers were removed quickly, weighed, immediately frozen on dry ice, and stored at −40°C until analyzed. Tyrosine aminotransferase activity was determined by Wurtman's modification (*2*) of the method of Diamondstone (*3*).

Tyrosine aminotransferase activity increases rapidly after birth (Fig. 1a). A recognizable periodicity, with a maximum at 10 a.m. and a minimum at 10 p.m., occurs within 48 hours after birth. At 22 days (Fig. 1b), the cycle is maintained, but its pattern is characteristic of the adult rat, with a maximum at 10 p.m. and a minimum at 10 a.m. The patterns of TT activity of rats weaned on day 21 and of those not weaned 21 to 23 days after birth were not significantly different.

The reversal from the neonatal pattern of periodicity to the adult cycle appears to occur between the 21st and 23rd day. The 10 a.m. activity, which is maximum in the early neonatal period, decreases about 50 percent. In contrast, the 10 p.m. activity, which is low during that early period, increases about 1.5 times by day 22. This trend continues, and when the rats are 3 months of age, the three- to fourfold difference (*1*) between maximum and minimum activity is observed (Fig. 1c).

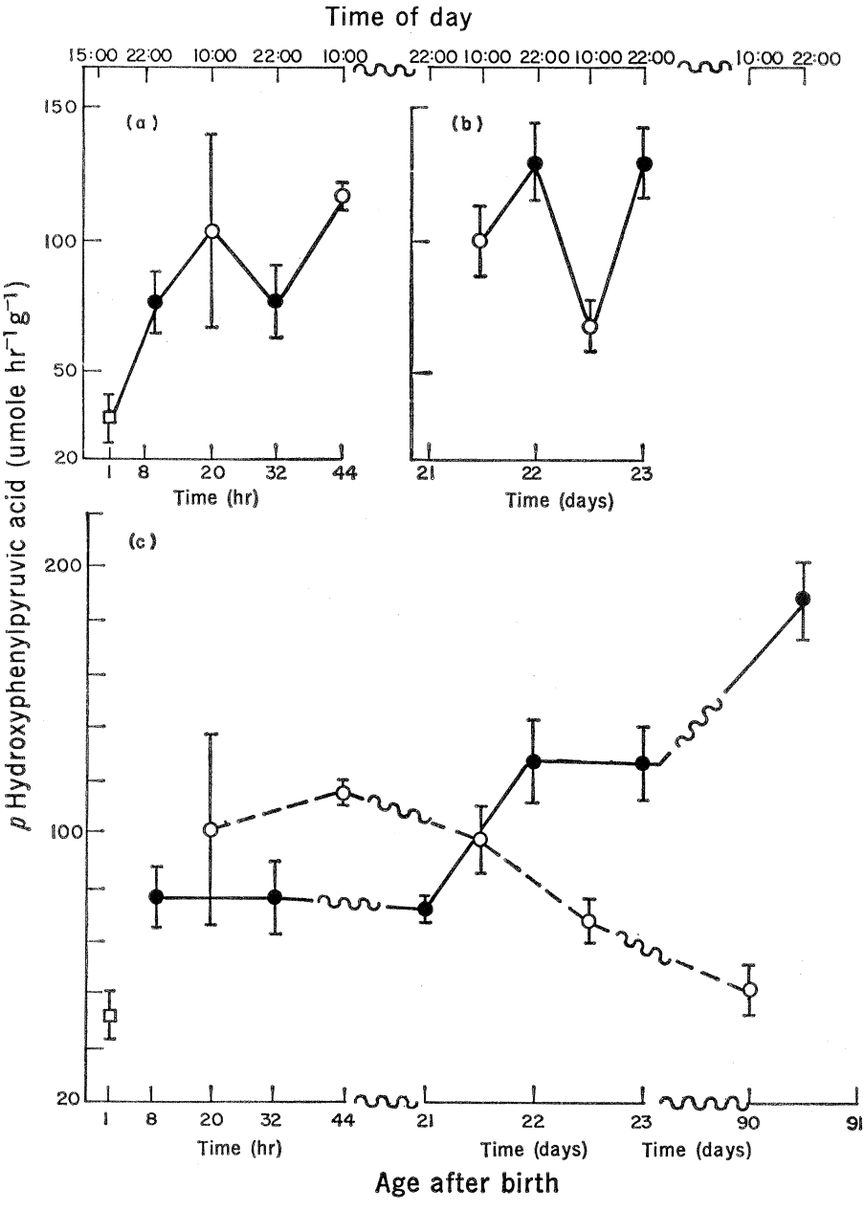

Fig. 1. Development of tyrosine transaminase activity in rats. Enzyme activity is expressed as the number of micromoles of *p*-hydroxyphenylpyruvic acid formed per hour per gram (wet weight) of liver. (a) Activity from 1 to 44 hours after birth. (b) Activity from 21 to 23 days after birth. (c) Comparison of the development of activity at 10 a.m. and at 10 p.m. from birth to 91 days. Closed circles represent activity at 10 p.m.; open circles represent activity at 10 a.m. In all these, values are expressed as the mean and standard error for each point.

No differences in the observed patterns could be determined when TT activity was calculated per gram of liver, per milligram of DNA, or per milligram of RNA (*4*). The variation in the enzyme's activity at each point in time is greatest when variation in the individual feeding patterns among animals is maximum. This occurs 20 hours after birth, when the young are beginning to suckle regularly, and during the hours after weaning. In both cases, the variation around each point decreases as more uniform feeding patterns evolve.

The generally increasing TT activity at 10 p.m. in the liver of the 3-week-old rat may be a function of the change from the diffuse feeding pattern of the newborn (*5*) to the more marked one of the adult (*2*), and also of the increased consumption of the casein agar-gel diet at the expense of the mother's milk. The increase in the proportion of protein in the diet, from 10 percent in rat's milk to 18 percent in the solid diet, may be especially relevant since TT activity appears to be related to the content of protein in the diet (*7*). On the other hand, a high carbohydrate diet has no effect, or at best depresses, the liver TT activity (*7*).

The rhythm of TT activity in the liver of the adult rat is independent of adrenal or hypophyseal activity (*1*), but it is generated by a period of significant intake of diet, especially of protein or its subunits (*1*, *2*). Thus, the cyclic changes of TT in the liver of the neonatal rat may be regulated in the same way, particularly since the interaction of hypophysis and adrenals is not fully developed in the newborn rat (*8*). It is possible that TT may demonstrate one of the earliest exogenously regulated enzyme rhythms appearing in the postnatal period.

It was reported that TT activity increased rapidly in the newborn, about 12 hours after birth reaching a value higher than that in the adult (*9*). Although our data for the first 20 hours after birth confirm this increase, a cyclic pattern of TT activity was observed during the first 48 hours. Thus, the decrease in TT activity from the early postnatal period to adulthood observed by other authors may be an artifact of sampling time in a cycling enzyme system, since the maximum

activity in the adult is higher than that in the young animal (Fig. 1c). The detection of a daily rhythmicity in rat liver TT activity so soon after birth emphasizes the extreme care with which future investigations of developmental changes in enzyme activity should be carried out.

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Estradiol-Concentrating Neurons: Topography in the Hypothalamus by Dry-Mount Autoradiography

Abstract. *Serial section autoradiograms were prepared of different planes of the hypothalamus and diencephalon of immature female, immature male, and ovariectomized mature rats injected with 6,7-^{3}H-estradiol-17 β. Known causes of diffusion and redistribution of the label, such as fixation, embedding, and thawing, were eliminated by the use of an autoradiographic technique based on the dry-mounting of freeze-dried sections. Neurons that concentrate estradiol exist in distinct and definable anatomical areas that are independent of the sex and hormonal state of the animals. Distribution of these neurons follows known terminations of the stria terminalis, which supports the concept of an endocrine amygdaloid-hypothalamic-hypophysial axis.*

Hohlweg and Junkmann (*1*) first provided evidence for neuronal control of pituitary function and postulated the existence of a "sexual center." Numerous attempts have since been made to determine its localization; the methods used include destruction of specified regions, stimulation, ovarian and hypophysial grafting, hormone crystal implantation, measuring of nuclear or nucleolar diameter of neurons, determination of regional uptake of radioactively labeled estrogens, and autoradiography. Although evidence suggests that the basal hypothalamus, the anterior hypothalamus, and the preoptic region influence gonadotropin concentrations (*2*), the precise anatomical representation of the areas involved is in question, largely because of limitations inherent in the technical approaches used, that is, lack of resolution, lack of differentiation between effects on cells or nerve-fibers, and diffusion of applied hormones in vivo or redistribution during tissue processing. The latter is most conspicuous in the controversial and misleading data obtained by autoradiography with ^{3}H-stilbestrol (*3*) and ^{3}H-estradiol (see *4*).

A precise anatomical determination of the areas involved in the feedback control, important for the understanding of the physiology and pathology of hormone regulation and the pharmacology of treatment with hormones and antihormones, particularly antifertility drugs, requires techniques that provide a cellular and subcellular resolution. Mapping of hormone-concentrating sites in the hypothalamus may also help to determine whether feedback control for different endocrine glands occurs in separate centers or in one nucleus.

The autoradiographic method used (*5*, *6*) was adapted for the study of brain tissue and excludes all the known sources of translocation artifact. It is based on (i) simultaneous freezing and mounting of small blocks of tissue (ii) low-temperature cryostatic sectioning and freeze-drying of 2.0-μ sections, and (iii) dry-mounting of the unfixed and unembedded sections on photographic emulsion (*6*).

Three immature female Sprague-Dawley (SD) rats, 25 or 29 days old, one immature male SD rat, 24 days old, and two ovariectomized mature female SD rats, 65 and 101 days old, ovariectomized 21 and 3 days, respectively, before the experiment, were injected with 6,7-^{3}H-estradiol-17β (*7*) (0.09 to 0.4 μg) dissolved in isotonic saline. The animals were decapitated 1 to 2 hours after the injection, the hypothalamus or diencephalon was excised and frozen, and 2-μ serial sections were cut in a cryostat at $-40°$ to $-50°$C. The sections were freeze-dried by cryosorption-pumping (*6*) and then dry-mounted onto photographic emulsion-coated slides (Kodak NTB 3) by pressure with a Teflon support. After exposure for 3 to 8 months at $-15°$C, the slides were processed and stained with methyl green–pyronin. Photomicrograms and sketches were prepared from microscopic slides and used for schematic drawings adapted to the sagittal and coronal planes of the atlas of König and Klippel (*8*).

In the ovariectomized rat, the autoradiograms encompass the hypothalamus, preoptic region, septal region, and anterior and medial thalamus; in the immature female and male, the studies were limited to the hypothalamus, the caudal half of the preoptic region, and the ventral thalamus. Radioactivity concentrated in nuclei of neurons (Figs. 1 and 2), but not in glial and ependymal cells, which are located in distinct and definable areas (Figs. 3 and 4); neurons of other areas remained unlabeled. In the hypothalamus and preoptic region studied the localizations of estradiol-concentrating neurons were not different in ovariectomized mature, immature female, and immature male rats.

Labeled neurons were concentrated in the nucleus arcuatus and the pars lateralis of the nucleus ventromedialis, including less densely packed neurons in its lateral and caudo-lateral vicinity. Krieg, who described five subunits of the nucleus ventromedialis in the rat, did not attach "especial significance to the differentiation of these parts" (*9*). His meticulous description, however, facilitates the recognition of the now apparent differences. The neurons of the nucleus hypothalamicus lateralis, characterized as large and stellate, with several long tapering processes (*9*), were not labeled. The nucleus periventricularis with its outgrowth of the nucleus paraventricularis parvicellularis contained neurons with a higher proportion of labeling in the anterior hypothalamic and preoptic region than in its more caudal part. The magnocellular portion of the nucleus paraventricularis remained, except for a few interspersed smaller neurons, unlabeled. A group of elongated, medium-sized cells located