Cyclic Fluctuations in I-131 Content of Thyroid Glands of Cats and Monkeys.* (31092)

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Diurnal cycles have been reported in various pituitary-target organ relationships(1). Evidence that the thyroid may be affected by such rhythms comes from the work of Walfish *et al*(2) who described cycles in the rate of degradation of exogenous thyroxin in humans, from that of Auerbach(3) who found similar variations in serum protein bound iodide and from the recent report by Bakke and Lawrence(1) that a circadian rhythm was observed in pituitary and serum concentration of thyroid stimulating hormone (TSH) in the rat.

We have obtained more direct evidence for cyclic thyroid function. Using the release curve method of Brown-Grant *et al*(4), we have observed rhythmic fluctuations in thyroid content of radioactive iodide (I-131) in cats and monkeys. In addition to the theoretical implications for the study of pituitary-thyroid function in general and of thyroid iodide metabolism in particular, these cycles are of considerable practical significance in analysis of the results of experiments in which thyroid content of iodide, in whatever form, is determined.

Materials and methods. Twelve adult female cats of mixed breeds and 2 female monkeys *(M. mulatta)* have been studied. Two of the cats were decerebrated, and in 2 the spinal cord was transected in the lower cervical region. In the 2 decerebrated cats the brainstem was divided at the hypothalamo-mesencephalic junction and there remained rostral to the transection only an island of tissue consisting of hypothalamus and pituitary. The surgical procedures and the pre- and postoperative care of the spinal and decerebrate cats have been described(5). Both of the monkeys and 9 of the cats had their thyroid glands transplanted to the tail. All surgical operations were performed several weeks or months prior to the experiments reported here.

The cats were housed in individual cages and except for the spinal and decerebrate ones they had free access to food and water. The 4 cats with central nervous system lesions were fed by stomach tube twice daily at approximately 0900 and 1700 hours. In all experiments except one, the cats were kept in a room in which temperature and lighting were controlled. The temperature was 26 ± 2°C and the lights were on 12 hours and off 12 hours. During each experiment the 2 monkeys were kept in restraining chairs in a room in which temperature and lighting were not controlled. At other times they were housed in individual cages in a large room with other monkeys.

For each experiment animals with transplanted thyroids received 5-15 μc I-131. The cats with *in situ* glands received 150 μc. In all cases the isotope was administered by intravenous or subcutaneous injection. Following injection of the isotope determinations of radioactivity in the region of the thyroid were made at 2-6-hour intervals. In some experiments the measurements were commenced immediately after injection; in others they were not started until after the time of maximum accumulation of isotope which is about 96 hours in the cat (Bard and Woods, unpublished data).

Scintillation detectors with 2″ NaI crystals and conventional amplifiers and scalers were used. Measurements of neck and thigh radioactivity were made using a Nuclear-Chicago flat field collimator; for tail measurements we used a lead shield with a tunnel through which the tail passed.

All measurements were corrected for physical decay and for tissue or environmental

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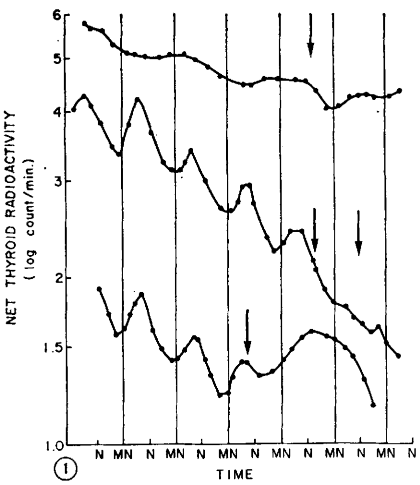

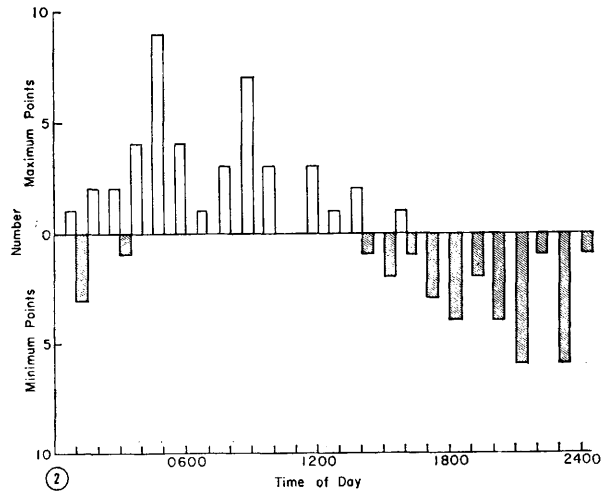

FIG. 1. Fluctuations in thyroid content of I-131 during 5-6 day periods. Top tracing: monkey #220, arrow marks onset of electrical stimulation of the brain (hypothalamus) which was continued for the remainder of the period shown. Middle tracing: spinal cat #90, arrows denote a 24 hr period of hypothermia (31-33°C). Lower tracing: cat #90, arrow marks injection of 25 μg. l-thyroxin. Abcissa: time, N = noon, M = midnight. Ordinate: arbitrary scale to fit in *all* 3 thyroid content of I-131 in cats.

FIG. 2. Bar graph showing hourly incidence of maximum and minimum points of cycles in thyroid content of I-131 in cats.

background. A segment of tail remote from the transplant was used for tissue background in the case of the animals with thyroid transplants. Tissue background for an *in situ* thyroid in a normal cat is difficult to obtain. In the past we have used measurement over the upper thigh as an approximation. A thigh count in a decerebrate or spinal cat can be made with ease; such a measurement in a normal cat requires patience and cooperation which is difficult to obtain. For this reason, in the experiments reported here measurements of neck radioactivity were not made during the first 4 days following injection of I-131. Past experience has shown that after 4 days thigh radioactivity would be no more than 2-3 times environmental background. In all the experiments reported here neck radioactivity was more than 50 times environmental.

Results. Examples of the cycles observed in cats and monkeys are shown in Fig. 1. The period of the cycle in cats varied between 20 and 30 hours. The cycle shown for the monkey in Fig. 1 had a period of about 48 hours; in other experiments shorter periods were found.

On the basis of 31 complete cycles in the 10 cats with intact brains, the average period was 24.8 $\pm$ 1.9 (S.D.) hours. In both species the period was relatively constant during any one experiment but it varied from one experiment to another. Peak radioactivity occurred in the morning (avg = 0714 hr) with minimum activity in the evening (avg = 1942 hr). The incidence of maximum and minimum points is shown in Fig. 2.

Cycles in the cats have been disrupted by injection of thyroid hormone and by hypothermia and in the monkey by electrical stimulation of the hypothalamus (Fig. 1).

In 3 experiments in the 2 decerebrate cats no cycles were observed (Fig. 3).

Discussion and conclusions. It may rightly be asked why it is that these cycles were not previously observed in the many laboratories including our own which have for several years used the release curve to detect changes in thyroid function. There are at least 3 explanations for our failure to recognize this phenomenon. First, the cycles were not detected until we set out to make more quantitative measurements by using transplanted thyroid glands. Second, measurements were formerly made between 0900 and 1000 hours and again between 1600 and 1700 hours

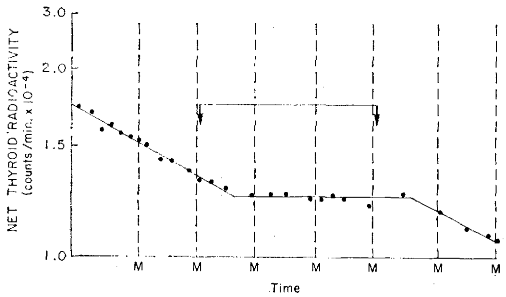

FIG. 3. I-131 content of the thyroid gland of a decerebrate cat with an isolated hypothalamo-pituitary island. Dashed verticle lines mark midnight on successive days. Arrows denote 72 hr period of hypothermia.

thereby missing the maxima and minima; and third, in many of the earlier experiments decerebrate cats with hypothalamo-pituitary islands were used.

The rationale for studying transplanted glands was that counting efficiency would be improved by the close proximity of the gland to the detector and that due to improved collimation the precision of the measurement would be greater. The data indicate that these assumptions were valid. The dose of I-131 necessary to yield a usable counting rate in the transplants is 1/20th to 1/10th that required by the *in situ* preparation and the sample/background ratio in the case of the transplants is several-fold that obtainable in intact cats.

After the cycles were observed in transplanted animals further measurements on intact cats were made. To obtain similar counting rates relatively large doses of radioactivity were required. Measurements made during uptake and early release phase were of doubtful consequence for the previously mentioned reason that reliable non-thyroid tissue background is unobtainable. Nevertheless, measurements made 5-8 days after injection show that the cycles are present in cats with intact thyroid glands, and it appears now that some earlier experiments were aborted due to what we labelled "unacceptable variations in counting technique" when we may have used good technique at an inappropriate time of day.

Bakke and Lawrence found in the rat that pituitary TSH content was lowest during the late morning hours when serum TSH reached its maximum value. It would not be profitable to attempt definitive correlations with their results due to the facts that different species are involved, the release curve has such poor time resolution and the relationship between plasma titer of TSH and thyroidal trapping of inorganic and secretion of hormonal iodide is incompletely known. However, their results and those recorded here are certainly not contradictory. The disappearance of radioactivity, presumably as labelled hormone, exceeds accumulation of inorganic ions at a time when Bakke and Lawrence found an increasing serum titer of TSH and accumulation outstrips secretion at the time when they found TSH concentration decreasing in the circulation and increasing in the pituitary gland. On the basis of available evidence regarding the dynamics of the release curve, low or declining TSH concentration would favor accumulation over secretion while rising or high titers would favor net secretion. In cats and rabbits a sudden increase in TSH brought about by injection of the hormone is followed after a short latency by a prolonged discharge of radioactivity(4, 5). Conversely, stoppage of TSH secretion by hypophysectomy or injection of thyroid hormone results in cessation of the discharge of radioactivity within a very short time, possibly minutes, while iodide uptake continues for a period of days(4,5,6,7).

If TSH concentration rises and falls rhythmically then it is to be expected that both rates—trapping and secretion—continually change. Since it may be reasonably assumed that both processes are affected unequally in both time and duration, the fluctuations in uptake and release are probably out of phase. If to measurements similar to those reported here are added simultaneous determinations of arterial and thyroid venous concentrations of radioactive inorganic and hormonal iodide, all of the data together should permit some deductions regarding the quantitative effects of TSH on intrathyroidal mechanisms and perhaps an elementary mathematical treatment of the rate constants involved in the trapping and secretion of iodide.

The amplitude of the cycle in cats such as that shown in Fig. 1 indicates that a very large fraction of the radioactivity lost in one phase of the cycle is reaccumulated in the next. This suggests that the rate of degradation of thyroid hormone in the cat is considerably higher than that which has been reported in other species. It also indicates that thyroid-renal sharing of iodide in the cat differs from that described for the rabbit by Brown-Grant *et al.* They found that approximately 90% of the iodide from degraded hormone was excreted by the kidneys in 24 hours. Clearly such a 9:1 ratio favoring renal excretion is impossible in the cat.

The longer cycle observed in the monkey may not be characteristic of this species. In fact, several complicating factors were involved. Neither temperature nor lighting was controlled, the animals were under some restraint throughout the experiments and at the beginning of each experiment they were introduced into a strange environment. It is of some interest, however, that a 2-day feeding cycle has been reported in another simian species(8).

Finally, if the fact that the 2 decerebrate cats did not show cycles proves to be the general case then it will be fair to conclude that the central nervous system participates in the timing if not the initiation of the rhythm. The site and nature of the central mechanism will be an interesting problem for future investigation. On the basis of present data the spinal cord below the 7th cervical segment may be excluded.

Summary. Cycles were observed in thyroid content of radioactive iodide in cats and monkeys. The period of the cycles in cats was approximately 24 hours. In the 2 monkeys it was longer. The cycles occurred in both species after transplantation of the thyroid and persisted in the cat following division of the spinal cord in the lower cervical region. In 3 experiments on 2 decerebrate cats no cycles were found.

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Occurrence of Adenovirus-SV40 Hybrids Among Monkey Kidney Cell Adapted Strains of Adenovirus.* (31093)

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In order to obtain adenovirus strains suitable for production of respiratory vaccines, field strains of adenovirus types 1 through 7 were adapted to rhesus monkey kidney (RhMK) tissue culture(1). It was recently found that the adenovirus 7 vaccine strain LLE46, which had become contaminated with SV40 virus during RhMK adaptation and been freed of the contamination by passage with SV40 antiserum, had formed an intergeneric "hybrid" with a portion of the SV40 genome (2-4). A proportion of the adenovirus capsids in this strain contained the SV40 genetic material which induced SV40 T antigen. The ability of the E46 virus to propagate in monkey cells was dependent on these SV40 DNA

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