hormones. For example, the temporal pattern of the rhythms of endogenous corticosterone (*10*) and prolactin (*11*) in photosensitive birds could be expected to favor gonadal growth. Consequently, there would be competition between the endogenous system and one applied by exogenous hormones in which the temporal pattern inhibits gonadal growth. Similarly, the rhythms of the hormones in photorefractory birds (*10, 11*) would inhibit gonadal growth and allow for a limited rate of increase in gonadal size even when the exogenous hormones are supplied in a temporal pattern favoring rapid gonadal development.

The possibility that the stresses incurred by handling may serve as *Zeitgebers*, and produce responses, appears unlikely. There were no responses among controls injected with saline. In addition, adrenal steroids entrain rhythms of fattening responses to prolactin in a fish, a lizard, and the common pigeon, and they entrain a daily rhythm of pigeon crop sac response to prolactin whereas saline injections do not (*9*). Our experience supports the evidence (*12*) that the adrenal steroids are important synchronizers of daily rhythms, including the daily rhythms of responses to prolactin.

The daily rhythms in the amounts of corticosterone in the plasma (*10*) and prolactin in the pituitary (*11*) of the white-throated sparrow are interesting in light of its rhythms of fattening and gonadal response. In May when birds are photosensitive, there is a 12-hour interval between the rise of plasma corticosterone and the release of pituitary prolactin; whereas in August when the birds are photorefractory, there is an interval of about 6 hours between the increase in plasma corticosterone and the release of prolactin. These findings corroborate our conclusion that the relations between the daily rhythms in plasma concentrations of corticosterone and prolactin have a role in organizing metabolic and reproductive conditions in some birds. Changes in the relations of the two hormone rhythms may account for the seasonal conditions of photosensitivity and photorefractoriness.

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Thyroxine: Conversion to Triiodothyronine by Isolated Perfused Rat Heart

Abstract. *Thyroxine labeled with carbon-14 and iodine-125 was perfused through surviving rat hearts. Only when unlabeled triiodothyronine was added as a carrier could the newly formed doubly labeled triiodothyronine be isolated. The fact that this triiodothyronine was labeled with the correct ratio of carbon-14 to iodine-125 indicated that it originated from thyroxine. Approximately 5 percent of the initial carbon-14 radioactivity was found in the recovered triiodothyronine.*

The conversion of thyroxine (T-4) to triiodothyronine (T-3) occurs in vitro (*1*) by removal of iodine from the β-ring. Triiodothyronine has been isolated from human plasma (*2, 3*). Since only part of the T-3 found in serum originates in the thyroid, the T-3 formed elsewhere becomes important for the evaluation of the total metabolic picture.

Some patients experience tachycardia when treated with T-4. Since T-3 is physiologically four to five times more potent than T-4 (*4*), it is possible that tachycardia may occur as a result of formation of T-3 by the heart. There has been no evidence for the direct conversion of T-4 to T-3 by the heart although evidence exists for such conversion in some tissues (*5*). Sterling and others have identified the presence of T-3 in human serum after T-4 therapy (*6–8*).

We have perfused surviving isolated rat hearts (*9, 10*) in investigating the possibility of this conversion. Perfusions were carried out at 37°C with 36 ml of Krebs Ringer buffer (*9*) containing [2-^{14}C; 3′,5′-^{125}I]thyroxine (50 μg/ml; 1 μc of ^{14}C and 1 μc of ^{125}I per milliliter). Attempts to isolate T-3 from samples of the circulating fluid obtained at regular intervals throughout the duration of the perfusion (90 minutes) yielded T-3 in only trace amounts. We used the analytical techniques of Sterling *et al.* (*3*). Preliminary chromatography on Dowex AG50 WX-2 was followed by paper chromatography with a mixture of hexane, *tert*-amyl alcohol, 2*N* NH_4OH (1 : 5 : 6 by volume). The procedures for protecting T-4, suggested by Kobayashi *et al.* (*11*), were used throughout this work.

There is evidence (*12*) that T-3 is rapidly metabolized by some tissues. Thus, it is possible that newly formed T-3 could be rapidly absorbed or degraded by the heart tissues (or both). This could account for the fact that only trace amounts of T-3 were isolated from the circulating fluid. To saturate the heart and thus reduce the chances of labeled T-3 being metabolized, we added unlabeled T-3 (1.0 mg) to the circulating perfusion medium to trap the labeled T-3 that might be formed. The hearts maintained approximately the same heartbeat [185 ± 30 (S.D.) beats per minute] when the new perfusion medium containing carrier T-3 was used.

When the modified perfusion solution containing carrier T-3 was used, it became possible to repeatedly isolate labeled T-3. This T-3 exhibited a ratio of ^{14}C to ^{125}I double that in the T-4 used initially; this finding conforms

with the expected result (*7*). The average percentage of T-3 found in the circulating fluid for four perfusions was 5.5 ± 1 of the [^{14}C]T-4. This amount was obtained almost immediately (5 minutes) and it only increased slightly during the remaining 90 minutes of the perfusion. Analysis of the labeled T-4's used in these experiments (New England Nuclear and Isolab, Inc.) indicated less than 0.1 percent of T-3 present as an impurity. Perfusion of the glassware with the doubly labeled T-4 and carrier T-3 in the absence of heart tissues yielded (after 90 minutes) only 0.1 to 0.2 percent of doubly labeled T-3. After the perfusions, the heart tissues contained over 45 to 55 percent of the ^{14}C and 40 to 50 percent of the ^{125}I in original solutions.

We conclude that the isolated surviving heart is capable of rapidly removing iodine from T-4. The exact amount of T-3 formed is difficult to determine because of the great amount of newly formed T-3 retained by heart fibers. The conversion of 5 to 6 percent of T-4 into T-3, as observed by our technique, would be an estimated lower limit of this activity.

Chromatography of the perfusing solutions indicated the liberation of free iodide (3 to 6 percent of the initial amount of ^{125}I). As calculated from the ^{14}C content, yields of 10 to 15 percent of tetraiodothyroacetic acid and 12 to 16 percent of mono- and diiodothyronine were obtained. Other iodinated materials were observed but not identified.

These results confirm the postulate of Sterling and others (*3, 7, 8*) that conversion to T-3 is an early step in the metabolism of T-4. The high activity of deiodinase in the heart may contribute to the tachycardia observed in some individuals.

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Nonencapsidated Infectious DNA of Adeno-Satellite Virus in Cells Coinfected with Herpesvirus

Abstract. *Adeno-associated satellite viruses produce antigen detectable by immunofluorescence but not infectious virus in tissue culture cells coinfected with herpes simplex virus. Analysis of DNA extracts from these infected cells shows that large amounts of infectious satellite virus DNA are produced but not encapsidated in the system. This result indicates that satellite virus may be defective at the maturation step.*

Adeno-associated satellite viruses are small, defective DNA viruses which produce infectious progeny only in cells coinfected with adenoviruses (*1*). Herpesvirus can provide a more limited helper function than competent adenoviruses (*2, 3*). Satellite viruses types 1 and 3 produce antigen detectable by immunofluorescence but not infectious virus or complement-fixing antigen in tissue culture cells coinfected with herpes simplex virus. No evidence was offered in these studies for synthesis of adeno-satellite DNA in these herpesvirus-satellite infected cells. The studies of Atchison (*2*) indicate that although no infectious satellite virus or even empty viral capsids are produced, abundant satellite virus structural antigens, presumably capsid proteins, are sequestered in the satellite-herpesvirus system. If some form of satellite virus DNA is replicated it is obviously not encapsidated and would therefore appear to be ideally suited for release from infected cells by mild extraction procedures.

We have recently studied the intracellular DNA species produced during satellite virus infection of green monkey cells in the presence of simian adenovirus SV15 and have developed an assay for satellite virus DNA which is capable of detecting 0.025 μg of infectious satellite DNA (*4*). Satellite virus DNA is infectious when the inoculum contains forms that are either double-stranded or single-stranded (mixtures of "plus" and "minus" strands) (*4*). Below we describe how these methods have been applied to demonstrating that infectious satellite virus DNA is indeed produced in cells coinfected with type 1 satellite virus and herpes simplex virus.

The simian adenovirus SV15 and adeno-satellite virus type 1 preparations used have been described previously (*5*). The satellite preparation was treated at 56°C for 15 minutes prior to use, to inactivate the adenovirus helper. The KOS strain of herpes simplex virus type 1 was used. Assay for competent satellite particles was performed on human embryonic kidney (HEK) cells by using adenovirus type 7, free of satellite, as previously described (*5*). Satellite antigen was assayed by the micro–complement fixation test (*6*). The isolation and assay of infectious satellite virus DNA was done on green monkey BSC-1 cells. To isolate satellite DNA, drained BSC-1 monolayers in 16-oz (473-ml) bottles were infected with herpesvirus and satellite at a multiplicity of infection of 10. After adsorption for 1 hour at 37°C, the cells were washed three times before maintenance medium was added.

Low molecular weight DNA was selectively extracted from control and infected cells 12 to 18 hours after infection, by using the sodium–dodecyl sulfate–1*M* sodium chloride precipitation technique of Hirt (*7*). The 1*M* NaCl-SDS fractionation procedure separates DNA molecules on the basis of their molecular weight. Thus cell DNA is precipitated while SV15 adenovirus