Seasonal Changes of Heart Rate-Temperature Relationships in Toads.* (31427)

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Seasonal changes of heart rate-temperature relationships in frog hearts have been reported by various investigators. Barcroft and Izquierdo(1) and Smith(2) found that hearts removed from winter frogs showed exponential relations with temperature, while hearts from summer frogs had linear relationships. Evidence of an endocrine origin for these seasonal differences was presented by Carter (3) who showed that addition of thyroxine to the perfusate changed winter heart rate-temperature relationships to the summer type. Stier and Taylor(4) administered whole frog pituitaries to female winter frogs. After ovulation had occurred, the intact hearts of pituitary treated (pithed) animals ceased beating at 35°C—a critical temperature that was typical of summer frogs. Hearts of untreated winter (pithed) frogs stopped beating at 26°C. They suggested that the whole pituitaries contained thyroid stimulating hormone which increased endogenous thyroid hormone secretion. These studies were all done on excised frog hearts, or on hearts of frogs whose brain and spinal cord had been pithed. It has now been possible to study heart rate-temperature relations throughout the year in an intact, unanesthetized amphibian.

Materials and methods. Fifty-five adult male toads (*Bufo fowleri*) from our colony, maintained at 22 ± 2°C throughout the year, were used in this investigation. They were fed mealworms (*Tenebrio molitor*) 3 times each week. All the toads reported here were from 3 collection periods (May and June, 1963, '64 and '65). Those studied during July and August were designated summer toads. Those used in temperature experiments during the following December and January were called winter toads. Some from the 1964 summer collection were also restudied during May and June of 1965.

Body (core) temperature was held constant during each recording period by way of a tubular heat interchanger through which water was circulated from a temperature regulated water bath. A confining unit, fashioned from a block of urethane foam, held the toad fully prone on the interchanger. A concavity, contoured to fit the body of each toad, was shaped with a small soldering iron. Tunnels were cut so that the toad's nostrils were exposed to the air. All surfaces of the sponge were sprayed with a water-proof silicone-resin preparation which minimized electrical short-circuiting. All surfaces of the brass tubes of the interchanger were covered with vinyl electrical insulating tape.

Rectal temperatures were measured by means of a 3 mm thermistor. Heart rates were monitored by a high-gain electrocardiographic unit using a urethane-sponge harness for holding the electrodes against the ventral body wall. The 2 electrodes were coils of 7-strand, 32 gauge tinned copper wire, one placed just behind the front limbs and one in front of the rear limbs. The sponge harness was wrapped around the trunk of the animal and held in place by monel metal staples.

To obtain a minimum scatter of heart rate values it was necessary to train each toad to his confining unit with the rectal thermistor and electrode harness in place. Each animal was set up daily for about 4 hours and body temperatures were increased, or decreased, by several increments during each training session. After one week, the trained toads exhibited only intermittent bursts of body movements when confined to their holders. During each period of movements, heart rates rose above the pre-movement value and then, during the subsequent period of quiescence, fell rapidly to a steady-state level. The rates used in this study were calculated from measurements of the time for 10, or 20, beats made

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during the latter halves of the periods of quiescence. The points plotted on the graphs are individual rates obtained at constant body temperature.

Results and discussion. Heart rate-temperature relationships were obtained from 30 trained, male summer toads. Fig. 1 presents the relationship that was usually obtained during July and August. Note the continuous linear function from 14 to 35°C with a 100% increase in heart rate for a rise of 10°C. In these studies, the body temperature was never allowed to drop below 14° or rise above 35°C, since our initial pilot experiments indicated that at these low and high body temperatures cardiac arrhythmias developed and, especially at temperatures above 35°C, irreversible damage to the nervous system and heart occurred.

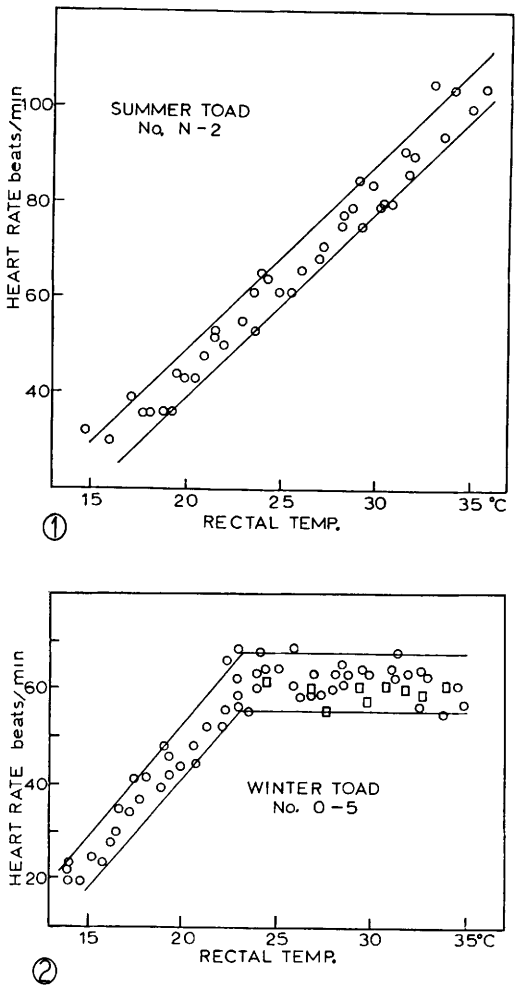

FIG. 1. Effect of temperature on heart rate of a male, unanesthetized, summer toad. Similar linear relationships throughout the range of 14-35°C were obtained from 30 male summer toads tested in this study.

FIG. 2. The heart rate-temperature relationship typically found in male winter toads. Twenty-five winter toads all had similar heart rate-temperature relationships. The linear relationship was obtained only over the range 14-23°C. Heart rates neither increased as body temperature was increased above 23°C (circles) nor decreased when temperature was decreased stepwise from 35 to 23°C (squares). Exponential heart rate-temperature relationships were never obtained from normal winter or summer toads.

During December and January the toads exhibited a different type of heart rate-temperature relationship. The linear relationship, exhibited by summer toads, held only throughout the range 14 to *ca.* 23°C. Also, at temperatures above 23° the heart rates did not increase as body temperatures were increased to *ca.* 35°C. Fig. 2 shows the typical temperature effects on heart rates exhibited by 25 trained, male winter toads. In this example, the heart rates did not increase as body temperatures were increased by small steps from 23 to 35°C nor did they decrease when the temperatures were then decreased from 35 to 23°C. The extreme variation of individual rates at each temperature was ± 10% of the mean throughout this upper range of body temperatures. The cardioregulation exhibited by winter toads in this upper range of temperatures is, presumably, a consequence of the state of the hormonal balance that existed during the winter months. We have not as yet attempted to change the winter type of temperature relationship into the summer type, or the summer type into the winter type, by experimentally altering the activities of the endocrine glands during each season. We have, however, restudied 3 of the winter toads during the following May and June. These animals gave typical summer type heart rate-temperature relationships.

Summary. Intact hearts of adult male toads (*Bufo fowleri*), collected locally in May and June ("summer toads"), gave linear heart rate-temperature relationships in the range 14-35°C. Toads from the same collection, tested in December and January ("winter toads"), also gave linear heart rate-tempera-

ture relationships, but only between 14 and 23°C. At temperatures between 23 and 35°C the heart rates of the winter toads did not increase when their body temperatures were increased. Seasonal differences in heart rate-temperature relationships had been reported for excised frog hearts and shown to be linked to seasonal changes in thyroid and anterior pituitary activities. The similar seasonal differences in heart rate-temperature relationships shown here in intact toads may also reflect endocrine changes. This working hypothesis remains to be tested directly.

1. Barcroft, J., Izquierdo, J. J., J. Physiol., 1931, v71, 145.
2. Smith, C. L., J. Exp. Biol., 1951, v28, 141.
3. Carter, G. S., ibid., 1933, v10, 256.
4. Stier, T. J. B., Taylor, Harriett, E., J. Cell. & Comp. Physiol., 1939, v14, 309.

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Purification and Toxicity of Aflatoxin G1.* (31428)

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There has been increasing recognition of the important role played by fungal metabolites as acute and chronic toxins in animals with possible implications in man. Attention has been focused particularly on the aflatoxins and much work has been done on their effects in animals(1,2) and on their chemistry. The structures of 4 of these coumarin derivatives have been established(3,4). In the course of our preparation of substantial quantities of some of these compounds from a mixture of aflatoxins derived from *Aspergillus parasiticus* grown on groundnuts, several interesting observations have been made and a reliable method for analyzing the crude mixtures has been developed.

Materials and methods. The aflatoxin mixtures (prepared by Dr. K. Sargeant, M.R.E., Porton) were resolved on thin layer (TLC) plates of silica gel H (Kensington Scientific Co., Oakland, Calif.) 20 × 20 cm, 1 mm thick for the preparative work, or of silica gel G, 5 × 20 cm, 0.5 mm thick for the analytical studies. The material was applied in chloroform solution as a thin streak 2 cm from the bottom of the plate. The developing solvent was chloroform-diethyl ether-acetic acid 2:2:1, which took a little more than 1 hour to ascend the plate; if separation of the components was not adequate, the plate was removed, allowed to dry for 30 minutes and redeveloped in the same solvent. At this stage the several fluorescent bands were separated by non-fluorescent zones. Each fluorescent band was scraped from the plate and placed in either a centrifuge tube or a flask (depending on the quantity of silica gel). For direct spectrometric estimation of the aflatoxins using standard absorptivity values (4) adsorbed fluorescent material was eluted with ethanol-chloroform 3:1. For preparation of aflatoxins the adsorbed material was eluted thrice with acetone-chloroform 1:1, followed by evaporation of the solvent. After repeated chromatography the separated components were sufficiently pure to be crystallized from chloroform by addition of methanol. The purity of each chystalline product was determined by analysis of 0.5 to 1 mg on a 5 × 20 cm TLC plate.

Acute toxicity determinations were made using white Pekin ducklings (average weight 57 g) collected from the breeders. These were given graded doses of the aflatoxins by mouth within 24 hours of hatching. The aflatoxins were dissolved in dimethylformamide (DMF), maximum volume 0.15 ml, and given by mouth before the ducklings were offered food. No deaths were recorded in control animals; the only effect was a diminished growth rate if the ducklings were given 0.20 ml or more DMF. At the doses used

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