should be sufficiently accurate to find site C by a difference Fourier map. The strong peak at site C in Fig. 1c appears with the expected reduction in peak height (*9*).

The heavy-atom parameters were refined to 6-Å resolution in the (*0kl*) projection with all coordinates constrained by $P6_222$ symmetry. The least-squares procedure and isomorphous phase calculation were essentially as described by Dickerson *et al.* (*10*); a program by Adams *et al.* (*11*) was used. A single site of 49 electrons gave refined coordinates of $x = .036$, $y = .476$, and $z = .091$, with a combined form-temperature factor of 278. Since more than 80 percent of the cell volume is solvent, it is reassuring that the osmium binding site is within the region indicated by various molecular search studies to be occupied by tRNA molecules (*4, 12*). The R factor:

$$\frac{\sum_h \left| \, |\Delta F(h)| - |F_{Os}^{calc}(h)| \, \right|}{\sum_h |\Delta F(h)|}$$

was 0.69, which is unexpectedly high in view of the close correlation between the E_Δ map of the (*0kl*) projection and the self-consistent interpretations of the various difference Patterson projections. The large residual may be due to subsidiary sites, because (i) certain peaks consistently recur in both the difference Fourier and Patterson projections, and (ii) there is only two-thirds occupancy of the primary site despite an overall stoichiometry of one osmium atom per tRNA molecule. Efforts to unambiguously establish the positions of subsidiary sites have not been successful in projection, and their determination awaits the analysis of the three-dimensional data.

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Brain and Body Temperatures in a Panting Lizard

Abstract. *Panting in* Sauromalus obesus *is effective enough to keep deep body temperature* (T_C) *and brain temperature* (T_B) *below an ambient temperature of 45°C for extended periods of time and has a greater cooling effect on the brain than on the remainder of the body. Six animals maintained* T_C *and* T_B *0.9°C (± 0.08 standard error) and 2.7°C (± 0.2 standard error) respectively lower than the ambient temperature of 45°C. It is possible that intracranial vascular shunts play a role in cranial cooling during panting.*

A number of investigators have pointed out the similarity between panting in homeothermic animals and the ventilatory response of certain desert lizards to heat stress, with the implication that the latter is a thermoregulatory response (*1*). Crawford and Kampe (*2*) suggested that the increase in respiratory ventilation could be due to the increase in oxygen consumption which occurs when the body temperature of a lizard is raised. They attempted to separate the respiratory response of the lizard *Sauromalus obesus* into its metabolic and possible thermoregulatory components. Although the respiratory pattern and evaporative cooling of *Sauromalus* at a body temperature of 43.5°C was consistent with the panting hypothesis, the animals did not dissipate heat sufficiently to maintain body temperature below that of the environment (43°C). Crawford and Kampe (*2*) therefore concluded that panting was of marginal importance in the overall heat balance of the animal.

It was necessary, in their experiments, to confine the animal in a double chamber which separated the head from the remainder of the body so that respiratory and cutaneous evaporation could be determined separately and simultaneously. Since it is unclear whether a seal separating the head from the remainder of the body interferes with respiration or gular flutter, it is desirable to determine whether *Sauromalus*, unencumbered by experimental apparatus, can maintain a body temperature less than that of the environment. Furthermore, the carotid arteries of *Sauromalus* run so close to the surface that they are visible in the pharynx, and they enter the cranium from an area exposed to air movement, particularly during gular flutter. It is possible that during panting and gular flutter evaporation from this area cools the carotid blood as it enters the cranial cavity, serving primarily to cool the brain rather than the entire body. Experiments were therefore designed to measure brain and deep body temperature simultaneously.

Animals (*S. obesus*) were anesthetized with tricaine methanesulfonate (MS-222; Ayerst), 400 mg/kg. A small hole, 1 mm in diameter, was drilled through the skull 1 to 2 mm behind the pineal organ. Membranes surrounding the brain were not penetrated by this procedure. The animals were allowed to recover overnight and appeared to be "normal" the following day. Brain thermocouples were prepared from 40-gauge copper and constantan wire. Thermocouples were passed through a 0.5-cm² Lucite plate, insulated and stiffened with a thin layer of dental acrylic, and implanted in unanesthetized lizards to a depth of about 0.5 cm. The position of the thermocouples, determined at autopsy, was at the rostral

border of the hypothalamus. To determine deep body temperature, thermocouples were passed through the cloaca into the intestine to a depth of about 5 cm. The animals were tethered to a wire frame which allowed free movement of the head and neck, and then placed in an incubator at 15°C. When temperature equilibrium was reached the animals were rapidly transferred to an incubator at 45°C and about 15 percent relative humidity, and heating curves were continuously recorded with a Honeywell Multipoint recorder for 2 to 8 hours.

The experiment shown in Fig. 1 reveals the time course of change in deep body temperature (T_C) and brain temperature (T_B) relative to an ambient temperature (T_A) of 45°C. Brain temperature increased more rapidly than body temperature (not shown in Fig. 1) until the animal began to pant at a brain temperature of about 39°C. After panting began, brain temperature leveled off and remained about 3°C below ambient during the 3.5-hour experiment. Deep body temperature, however, continued to increase and stabilized at about 1°C below ambient after 2 hours of exposure. This pattern of heating was consistent in the six animals tested. The mean temperature at which the heating curves crossed was 39.6°C (± 1.11 S.E.) and occurred several minutes after the onset of panting. The animals maintained a mean temperature difference between T_B and T_A of 2.7°C (± 0.24 S.E.) and between T_C and T_A of 0.9°C (± 0.08 S.E.). One experiment was allowed to continue for 8 hours and the animal was successful in maintaining brain and deep body temperature about 3°C and 2°C below ambient, respectively. Furthermore, when the mouth of a panting lizard was taped shut T_B equilibrated with T_C and together they approached T_A. When the adhesive was removed the original temperature differences were reestablished. When animals were heated to 35°C, T_B equilibrated with T_C at about 0.5°C above ambient temperature.

Four animals were killed after heating experiments with intraperitoneal injection of sodium pentobarbital, equilibrated at 15°C, and returned to the incubator at 45°C. The brain temperature of dead animals increased more rapidly than deep body temperature, and the heating curves came to equilibrium about 0.25°C below ambient temperature (Fig. 2). Failure of T_B and T_C to equilibrate with T_A can be attributed to continued evaporation from the dead (nonmetabolizing) animal.

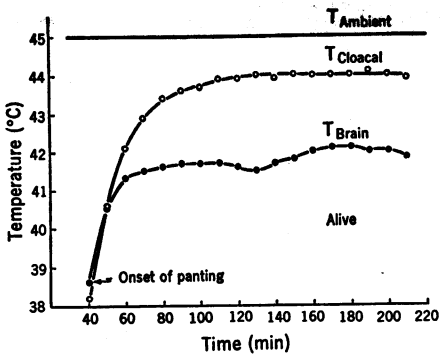

Fig. 1. Change in deep body temperature (T_C) and brain temperature (T_B) with respect to time (minutes). T_C and T_B were equilibrated at 15°C, then the animal was rapidly transferred to an incubator at 45°C (T_A). Initially T_B increased more rapidly than T_C.

The results indicate that panting in *S. obesus*, under these experimental conditions, is effective enough to keep T_B and T_C below 45°C for extended periods, and that it has a relatively greater cooling effect on the brain than on deep body temperature. In similar experiments on the desert iguana, *Dipsosaurus*, Templeton (*3*) found that cranial and cloacal temperatures came to equilibrium about 0.2°C above an ambient temperature of 46°C and thereafter never differed by more than 0.1°C. Furthermore, cloacal temperature increased more rapidly than cranial temperature. Templeton therefore concluded that no local cooling of the brain occurred in *Dipsosaurus* owing to evaporation of water from the buccal cavity, even though the animals were panting. It is unclear whether the difference between *Sauromalus* and *Dipsosaurus* is due to species variation, since in Templeton's experiments the animals were confined in chambers and it is uncertain whether the chamber humidity was low enough to permit sufficient evaporative cooling. DeWitt (*4*), however, observed that when *Dipsosaurus* was placed in an air flow at 50°C and a velocity of about 400 ft/min (120 m/min), body and hypothalamic temperatures leveled off at 3°C and 6°C, respectively, below air temperature. These temperature differences were maintained for at least 25 minutes. On the other hand, under field conditions brain and body temperatures did not differ by more than 1°C.

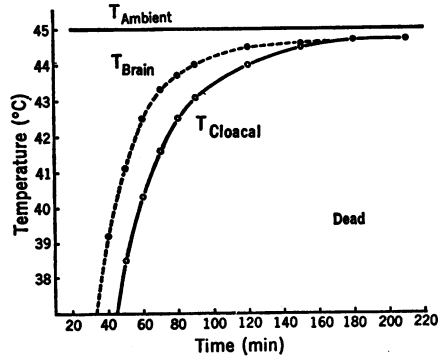

Fig. 2. Change in deep body temperature (T_C) and brain temperature (T_B) when a dead animal was transferred from 15°C to 45°C (T_A). Failure of T_C and T_B to equilibrate completely with T_A during the experiment was apparently due to continued evaporation from the dead (nonmetabolizing) animal.

Whether the brain-body temperature differences observed in *Sauromalus* during panting is coincident with intracranial vascular shunts, as occurs in certain mammals, is not known. Heat could be transmitted by conduction through the floor of the skull to the membranes covering the roof of the buccal cavity, a distance of several millimeters. Heath (*5*) has shown that during basking the brain temperature (middle ear temperature) of the horned lizard, *Phrynosoma cornutum*, increases faster than cloacal temperature up to about 36°C, where the head-body gradient disappeared. Disappearance of the gradient was associated with eye-bulging, apparently resulting from intracranial pressure changes. Heath (*6*) suggests that during basking, because of its large surface-to-volume ratio, the head heats faster than the remainder of the body. Warm blood from the cephalic venous sinuses flows through the internal jugular veins countercurrent to the cooler blood in the internal carotid arteries. Heat transfer from the warm venous blood to the cool arterial blood assists in the development and maintenance of the head-body temperature gradient. Heath proposes that when body temperatures reach about 30°C contraction of the internal jugular constrictor muscles increases cephalic venous pressure which opens lateral shunts from the cephalic sinuses to the external jugular and vertebral veins. Elimination of countercurrent heat exchange results in increased convective heat flow from head to body, thus diminishing the gradient.

If panting in *Sauromalus* cooled the carotid arterial blood, and if warm cephalic venous blood returned to the body primarily through the internal jugular veins countercurrent to the carotid arteries, the cool arterial blood entering the head would gain heat from the warm venous blood leaving the head. This countercurrent heat exchange would decrease the efficiency of panting in cooling the brain. On the other hand, if blood flow through the internal jug-

ular were occluded, eliminating countercurrent heat exchange, the cool carotid blood would gain heat from the surrounding brain tissue rather than jugular venous blood, thus cooling the brain. Heath (*6*) associated internal jugular occlusion with increased cephalic venous pressure, eye-bulging, and diminished head-to-body temperature gradient. DeWitt (*4*) observed that when the head of *Dipsosaurus* was heated the eyes protruded. He suggested that *Dipsosaurus* employed the same mechanism as *Phrynosoma* for equalizing head and body temperatures. Constriction of the internal jugular muscle at eccritic temperatures may result in the disappearance of the head-to-body temperature gradient. However, its constriction during panting could result in reversal of the gradient, keeping the brain cool relative to the rest of the body. It is possible, then, that the internal jugular constrictor muscle plays a more general thermoregulatory role in lizards than equalizing head and body temperatures.

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4 April 1972

Tumor Immunity in vitro: Destruction of a Mouse Ascites Tumor through a Cycling Pathway

Abstract. *Lymphocytes in peritoneal exudate from BALB/c mice immunized against ascites leukemia EL4 are uniquely efficient at destroying ^{51}chromium-labeled EL4 cells in vitro. The lytic process depends upon the number of lymphocyte–tumor cell interactions. Effector lymphocytes are not inactivated as a result of lethal contact but can interact repeatedly with tumor cells.*

Lymphoid cells from an animal immunized against a tumor can bring about the destruction of the tumor in vitro. This immune process is believed to be analogous to cell-mediated tumor regression in vivo (*1*, *2*). In an attempt to elucidate the cell mechanism involved in tumor rejection, we studied in vitro the destruction of tumor cells by lymphocytes that had been anatomically associated with the rejection of an ascites tumor in vivo (*3*). We report that effector lymphocytes are not inactivated as a result of lytic contact with tumor cells but can recycle to interact with and destroy additional tumor cells. The rate of tumor cell destruction depends upon the number of lymphocyte-tumor cell interactions.

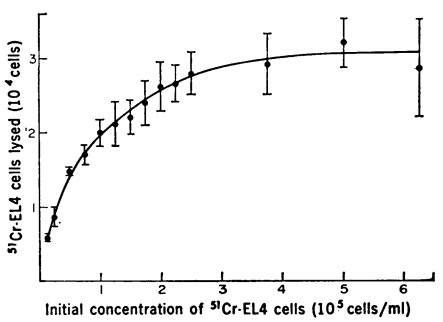

Fig. 1. Lysis of ^{51}Cr-EL4 cells as a function of ^{51}Cr-EL4 cell concentration. Lymphocytes (1×10^5) from BALB/c mice immunized with EL4 cells were mixed with the indicated concentrations of ^{51}Cr-EL4 cells and incubated while rocked for 45 minutes at 37°C. Control cultures contained normal BALB/c peritoneal lymphocytes under the same conditions. Results given were obtained by subtracting the means of triplicate control cultures from means of triplicate experimental cultures. Vertical bars indicate ranges.

Lymphoid cells were obtained from the peritoneal exudate of BALB/c mice 11 days after an intraperitoneal injection of 3×10^7 EL4 cells (*4*). The crude peritoneal exudate, consisting primarily of macrophages and small- and medium-sized lymphocytes, was passed through nylon wool columns to remove macrophages. The eluted nonadherent lymphoid cells (lymphocytes) were extremely effective at destroying tumor cells in vitro (*3*). In the cytotoxicity measurements, unlabeled lymphocytes and EL4 cells labeled with sodium [^{51}Cr]chromate (^{51}Cr-EL4 cells) were incubated in 1-ml portions of medium containing 10 percent fetal calf serum (*5*). The mixtures were rocked (5 cycle/min) at 37°C, and then the amount of radioactivity released was measured (*2*, *6*). The number of lysed ^{51}Cr-EL4 cells was determined by comparing the radioactivity released into culture supernatants with that obtained by freezing and thawing a known number of ^{51}Cr-EL4 cells.

To determine whether tumor cell destruction depended on the number of interactions between lymphocytes and tumor cells, and also to determine the subsequent fate of the lymphocytes, the number of tumor cells destroyed by a given number of lymphoid cells was measured.

In the first set of experiments, a constant number of immune lymphocytes was mixed with increasing numbers of ^{51}Cr-EL4 cells, and the number of tumor cells lysed was determined. In a 45-minute incubation, the number of ^{51}Cr-EL4 cells lysed increased as a function of the initial concentration of tumor cells (Fig. 1). However, the number of lysed cells reached a plateau when the initial concentration of ^{51}Cr-EL4 cells was 3×10^5 ml^{-1}. The number of ^{51}Cr-EL4 cells destroyed at this point (about 3×10^4) was the maximum number that could be lysed with the time interval and cell concentrations used (*7*). This number, however, did not reflect the total lytic capacity of the lymphocytes, because longer incubation resulted in more killing (Fig.

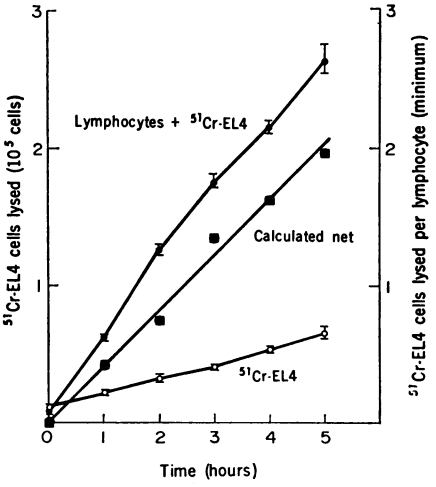

Fig. 2. Killing capacity of lymphocytes from BALB/c mice immunized with EL4 cells. Immune lymphocytes (1×10^5) were mixed with ^{51}Cr-EL4 cells (8×10^5) and incubated while rocked at 37°C for the times indicated. Results are means of quadruplicate cultures, and the vertical bars indicate ranges. The net number of ^{51}Cr-EL4 cells lysed (■) was calculated by subtracting mean control values (○, ^{51}Cr-EL4 cells) from mean experimental values (●, lymphocytes + ^{51}Cr-EL4 cells).