Table 1. Incorporation of glucose-C^{14} into $C^{14}O_2$ and C^{14}-lactic acid by corneal epithelium. The values are given as the mean ± the standard error in counts per minute, per gram of wet weight, per 3 hr. The specific activity of 1-C^{14} and 6-C^{14} glucose was 480 counts/min μmole.

Determinations		Total counts/min recovered		Ratio C-1/C-6
Compound	No.	1-C^{14} glucose	6-C^{14} glucose	
CO_2	12	3370 ± 68	507 ± 15	6.7
Lactic acid	6	5330 ± 221	8105 ± 204	0.65
Sum		8700	8612	

Bovine corneal epithelium (700 to 800 mg) was incubated for 3 hr at 37.5°C with 20 μmole/ml of glucose in 6.0 ml of Krebs-Ringer bicarbonate buffer equilibrated with a gas phase of 95-percent O_2 and 5-percent CO_2. At the end of the incubation period the reaction mixture, contained in an erlenmeyer flask equipped with a side arm and center well, was acidified, and the CO_2 released was absorbed by a sodium hydroxide solution injected into the center well.

Aliquots of the CO_2 recovered were analyzed for CO_2 content (*6*) and radioactivity. The radioactivity of CO_2 was counted as $BaCO_3$ with an end-window Geiger tube. The specific activity of 1-C^{14} and 6-C^{14} glucose was 480 counts/min μmole and was determined by the combustion method of Van Slyke and Folch (*7*). The amount of lactic acid recovered after incubation was measured by the method of Barker and Summerson (*8*). The specific activity of the lactate was determined after oxidation to acetaldehyde and was counted as the dimedon-acetaldehyde complex (*9*). To employ this method it was necessary to remove glucose from the reaction mixture. This was accomplished by deproteinizing the reaction mixture with 10-percent trichloroacetic acid.

The resulting supernatant, neutralized to *p*H 5 to 6, was then placed on a Dowex 1 (chloride) column 30 by 1.2 cm. The column was washed with 1 lit of water to remove all traces of the isotopic glucose. Upon subsequent elution with 0.01*N* HCl, the lactic acid was recovered within a 50-ml fraction, and without further treatment it was oxidized according to the method of Brin (*9*).

The reaction mixtures from two experiments were usually pooled to give a sufficient amount of lactate (60 μmole) for this determination. As a control of this method, nonradioactive lactate was separated from a simulated reaction mixture containing isotopic glucose, and no radioactivity was detectable when the recovered lactate was converted to the dimedon-acetaldehyde complex.

The results of the experiment when corneal epithelium was incubated with either of the two isotopic forms of glucose are shown in Table 1.

As is shown in Table 1, the ratio of $C^{14}O_2$ from 1-C^{14} glucose/6-C^{14} glucose of 6.7 indicates that there does occur a preferential cleavage of the C-1 of glucose in contrast to C-6. If glucose were metabolized via the glycolytic and citric acid pathways exclusively, a ratio of 1 would have been observed. It appears that the shunt mechanism is particularly active in the production of CO_2 in this tissue. That this is the case, even though most of the glucose is metabolized via the glycolytic scheme, suggests that the citric acid cycle is relatively inactive in this tissue.

From the amount of radioactivity incorporated into lactic acid, as shown in Table 1, it is apparent that there exists an active glycolytic pathway, and an approximation of the relative extent of this route and the shunt mechanism is possible. Since the C-1 of glucose is oxidized directly to carbon dioxide in the shunt mechanism, it would appear that the 5330 counts/min recovered in the lactate from 1-C^{14} glucose is derived solely by the glycolytic route. An identical amount of radioactivity in lactate must be furnished by glycolysis when 6-C^{14} glucose is the substrate, and the additional amount observed is that derived from the direct oxidative pathway. Therefore, the ratio of C^{14}-lactate from 1-C^{14} glucose/6-C^{14} glucose of 0.65 can be taken as the approximate fraction of glucose metabolized via the glycolytic pathway. It appears that about 65 percent of the glucose is metabolized via the conventional glycolytic scheme and 35 percent by the shunt mechanism.

It is of interest to note that the sum of radioactivity incorporated into carbon dioxide and lactic acid is identical from the two forms of labeled glucose. This indicates that in this tissue, the intermediates of the direct oxidative pathway do not accumulate to any appreciable extent. Furthermore, it must mean that the triose phosphates formed in the shunt mechanism are eventually converted to lactic acid.

In the corneal epithelium, even though most of the glucose is metabolized by the glycolytic pathway, the presence of the hexose monophosphate shunt is indicated because a preferential oxidation of the C-1 of glucose occurs. Moreover, since the citric acid cycle appears sluggish, the shunt mechanism probably plays a more conspicuous role in the production of biological energy in the cornea than it does in other mammalian tissues.

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8 April 1955

Acclimation of the Critical Thermal Maximum of the Reptile Urosaurus ornatus

Experiments were designed for preliminary investigation of acclimation of temperature tolerance in a reptile, the small lizard *Urosaurus ornatus linearis* Baird. The relatively small sample sizes ($N = 12$–15) have proved adequate. A total of 45 individuals was used, including young and adults in the body-weight range of 1.26 to 4.85 g. Measurements were restricted to those for the upper lethal temperature range.

In such investigation it is useful to explore two different but related expressions of temperature tolerance—that is, the critical thermal maximum, or incipient upper lethal temperature, and the resistance time. General methods currently used for determining such values for fishes were not employed (*1*). In the present work, the critical thermal maximum was first determined (= 43.1 ± 0.25°C); afterward the resistance time at 44.0°C was measured. Resistance time at a given constant temperature may be given as the arithmetic mean, or the geometric mean, survival time. The animals were tested singly in air in 100-ml-capacity glass tubes, immersed in an electrically heated and stirred water bath, with air supplied through coils of immersed copper tubing. In tests for the critical thermal maximum, the animals were started at 39°C and the temperature was raised 0.5°C every 30 min. The animal's body temperature reached the new environmental

temperature within 5 min of the change of bath temperature. Fresh animals from the field (field controls) were maintained 1 to 2 days at room temperature (22° to 26°C) prior to testing. Experimental animals were kept at the constant temperature of 35°C for 7 to 9 days (the acclimation period used) prior to testing. All lizards were collected in Sabino Canyon, near Tucson, Ariz., during April and May.

The critical thermal point of locomotory disability in *U. ornatus* under such experimental conditions is readily observed, in order to allow a respectable degree of accuracy for determination of the critical thermal maximum for the individual. The concept of the critical maximum and critical minimum of Cowles and Bogert (*3*) for a population may be modified so that either measurement may be visualized as a value that is the arithmetic mean of the collective thermal points at which locomotory activity becomes disorganized and the animal loses its ability to escape from conditions that will promptly lead its death.

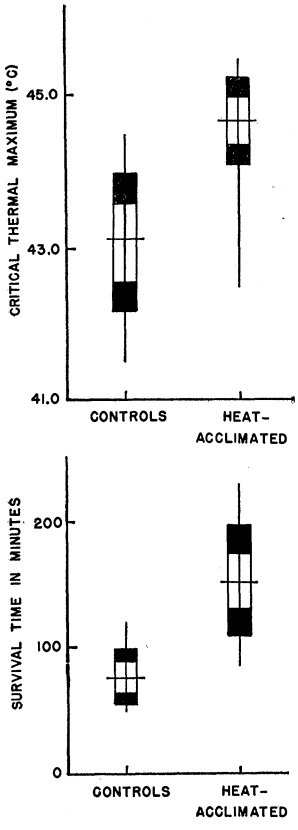

Fig. 1. Critical thermal maxima (°C) and resistance times (minutes) for the lizard *U. ornatus linearis* acclimated for 7 to 9 days at 35°C. The significant differences are shown graphically by the wide spread, without overlap, of the white rectangles, which represent values of two standard errors on each side of the mean. One black and one white rectangle combined, on each side of the mean, represents one standard deviation.

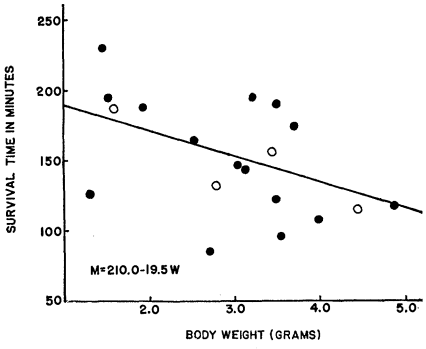

Fig. 2. Correlation of survival time with body weight for *U. ornatus linearis*. The four open circles represent the observations (black dots) grouped with class interval widths of 0.5 g, yielding a regression coefficient of $M = 215.7 - 22.6W$, which is not significantly different from the regression coefficient of the unclassed observations.

From the ecological and evolutionary point of view this *is* the lethal "point."

The means and their standard errors for the determinations of the critical thermal maximum in degrees centigrade, and their associated values of t and P, are as follows: Heat acclimated sample —44.5 ± 0.17; controls—43.1 ± 0.25; $t = 5.6$, $P < 0.001$ (Fig. 1).

The means, standard errors, t and P for the determinations of resistance time in minutes are as follows: Heat acclimated sample—152.8 ± 11.3; controls—76.4 ± 6.7; $t = 5.8$, $P < 0.001$ (Fig. 1).

The fact of a well-marked acclimation of the critical thermal maximum of *U. ornatus* is clearly established by both sets of data, which are graphed in Fig. 1. It may be seen that there is a direct, rather than an inverse, relationship between the direction of acclimation change and the acclimating temperature. The acclimating temperature used (35°C) is the mean of the normal activity range (eccritic mean) determined by us for the subspecies used (*3*).

Figure 2 depicts the inverse relationship between resistance time and body size (and age) for acclimated animals. The smaller (younger) individuals show greater resistance when subjected to relatively high environmental and body temperature. Moreover, under the experimental conditions employed, the small individual is at a given body temperature slightly longer than the larger individual, because of the more rapid heating of the smaller individual as a result of its greater surface-to-volume ratio.

The regression lines in Fig. 2 were fitted by the method of least squares. A hyperbolic curve has not been fitted, simply because of the scatter distribution of the small sample ($N = 15$). The correlation coefficient r is -0.474 for the individual ungrouped plots; the correlation is significant. For the same data (Fig. 2), when they are grouped with class interval widths of 0.5 g, r becomes -0.870, which is significant.

It has thus been demonstrated (i) that the critical thermal maximum of the lizard *U. ornatus* is significantly modified by acclimation to temperature, (ii) that the relationship between acclimation and acclimating temperatures is direct rather than inverse, and (iii) that the resistance to heat death is greater in the smaller, younger individuals.

After 7 to 9 days acclimation at a constant temperature of 35°C, the critical thermal maximum was increased by 1.4°C, and the resistance time at 44°C was approximately doubled.

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Effect of N-m-Tolylphthalamic Acid on Tomato Flower Formation

The *N*-arylphthalamic acids were first described by Hoffman and Smith [*Science* **109**, 588 (1949)] with regard to their influence in promoting fruit set in the tomato. We have subsequently found *N-m*-tolylphthalamic acid of practical value for promoting early fruit set of field-grown tomatoes when cool nights often prevent normal pollination of the first flowers.

In the spring of 1954, this chemical at a concentration of 200 parts per million (ppm) was applied to the foliage of eight tomato varieties in an attempt to promote early fruit set under greenhouse conditions. Three consecutive sprays were applied at approximately 2-wk intervals at anthesis of each of the first three clusters. Since environmental conditions were favorable, no influence of the chemical on fruit set was noted. However, it was observed later that the number of flowers, beginning with the fourth cluster of treated plants, was strikingly increased. This increase in flowers was accompanied by greater numbers of fruit on the fourth to seventh clusters (Table 1).

A field study during the summer of 1954 on 40 tomato varieties included both determinate and indeterminate types. During flowering of the first clus-