

STUDIES ON TEMPERATURE REGULATION

II. AMPHIBIA AND REPTILES

R. L. KIRK AND LANCELOT HOGBEN, F.R.S.

(Received 8 January 1946)

(With Four Text-figures)

1. INTRODUCTION

A previous communication (Hogben & Kirk, 1944*b*) drew attention to the fact that emergence of animal life on land offers a choice between two ways of solving a dilemma which arises as soon as animals expose themselves to climatic conditions of terrestrial habitats under adverse conditions of high air temperature and intense solar radiation:

(a) maintenance of body temperature below the lethal limit by evaporation of water from the surface;

(b) selection of stocks with a relatively high thermal death-point, hence able to penetrate dry land without restrictions imposed by such continuous obligatory loss of water.

Several authors (Gray, 1928; Hall & Root, 1930) have suggested that different rates of water loss, such as occur respectively among Amphibia and reptiles, furnish an important clue to this aspect of the evolution of terrestrial vertebrates. From their own observations Hall & Root conclude that the influence of external humidity changes on the body temperature of vertebrates (salamander, frog, toad, lizard, *Phrynosoma*, turtle and alligator) decreases as we pass from representatives of older to representatives of more recent groups; but none of these authors record how far changes of thermal death-point have accompanied increased resistance to desiccation. On some matters our own observations do not tally with those of Hall & Root on the body temperature of the frog (Hogben & Kirk, 1944*a*), and if the discrepancies are attributable to the apparatus they employed, similar observations carried out on other species merit reinvestigation. We have, therefore, felt it necessary to extend our investigations of the thermal death-point and body temperature of the frog (Hogben & Kirk, 1944*a*) to the toad (*Bufo bufo*), which is less restricted to a moist habitat, and to two representatives of reptilian stock, the alligator (*A. mississippiensis*) and the slow worm (*Anguis fragilis*). Since little is known about the effect of acclimatization on the thermal death-point of vertebrates it was felt advisable to confine our studies on thermal death to species indigenous to this country, and preferably to carry out experiments at the same season of the year.

The number of reptile species easily obtainable is thus strictly limited. Of the two stocks we have kept (*Lacerta vivipara* and *Anguis fragilis*) the former suffered such a high natural mortality under laboratory conditions as to make assessment of its thermal death-point without recourse to a very large number of animals open to doubt. The slow worm, however, is easy to keep in the laboratory, and is extremely viable even when starved for long periods. Out of a stock of 54 animals only 1 died during the course of 2 months.

2. METHODS

Two types of experimental chamber were constructed for the temperature investigations recorded below. The first, essentially the same as one previously described (Hogben & Kirk, 1944*b*), was used for the experiments on the toads and slow worms. A much larger chamber was constructed for the alligators. A square galvanized iron tank (37 × 80 × 89 cm.) was fitted with a lid, half wood and half glass to facilitate inspection of the animal during the course of an experiment. The tank was heated with a 1 kW. electric fire element, so arranged with baffles that no direct radiation fell on the animal; cooling pipes from a refrigerating unit were fitted into one corner. Humidity was controlled by means of trays of CaCl₂, water and H₂O : H₂SO₄ mixtures in the bottom of the tank, and the air was kept in rapid motion by means of a fan attached to the lid. To prevent excessive movement during an experiment the alligator was placed in a wide-mesh wire cage held on two supports inside the chamber so that air could circulate freely round the whole body of the animal. Conduction from the substratum was thus reduced to a minimum. Air temperatures, wet-bulb temperatures and body temperatures were measured with needle thermocouples manufactured by the Cambridge Instrument Company. For the purpose of measuring body temperature the thermocouple was driven into the musculature between the scutes about 2 in. posterior to the cloaca. Damage to the visceral organs was thereby avoided whilst permitting a fair assessment of the temperature of the main body region.

Special rectal thermocouples were constructed for the measurement of the body temperature of the slow worms. A copper-constantan junction (25 and 28½ s.w.g. respectively) was cemented with soft solder into one end of a silver tube of external diameter 0.3 cm. and length 4 cm. The leads ran back through the tube and were encased in thin rubber drainage tubing which was slipped over the other end of the silver tube. A cold junction was made closely identical to the two rectal thermocouples. They were used in conjunction with a high sensitivity short period galvanometer which was critically damped. The circuit was far more stable than with any of the types of thermocouple previously employed and readings accurate to 0.1°C. could be repeated after long intervals. During an experiment one of the thermocouples was introduced into the rectum of the slow worm and kept in position by binding the lead to the tail with cotton immediately behind the cloaca. The animal was then allowed to move freely around the experimental chamber moving over the surface of a perforated zinc plate raised 2 in. from the bottom of the chamber. The air temperature at the surface of the plate was measured with the other silver thermocouple and the wet-bulb temperature of the circulating air just above the surface of the plate determined by a mercury in glass thermometer graduated with divisions of 0.1°C., the bulb of which was surrounded with a wad of wet cotton-wool. Humidity control was effected by means of trays of H₂O : H₂SO₄ mixtures in the bottom of the experimental chamber and in the large air reservoir from which air was drawn by the circulating fan.

3. TEMPERATURE REGULATION OF AMPHIBIA

(a) *Thermal death-point.* The mortality of toads (*Bufo bufo*), obtained from dealers, was investigated by allowing them to swim in water for a period of 1 hr., the temperature of the water being accurately controlled to ±0.1°C. After immersion the toads were placed in clean moist dishes kept at room temperature and the mortality at the end of 24 hr. was noted. The body temperature during the period of immersion was taken to be the same as that of the water. In all, 95 toads were used and the results of these experiments are recorded in Table 1. It would appear from an examination of this table that there is little difference between the thermal death-point of *B. bufo* and that which we had previously determined for *Rana temporaria*. A further series of observations on *R. temporaria* was carried out at the higher temperatures. Combined with previous observations this provides the material for Table 2.

Under the experimental conditions described above it has not been possible to keep frogs of the species *R. temporaria* alive after immersion for 1 hr. in water at 33.5°C. An appreciable number of toads

Table 1. *April*
Temperature operative period 1 hr. Examined 24 hr. later

Temp. of water °C.	No. alive after 24 hr.	No. dead after 24 hr.
27.7	4	0
30.0	4	0
31.5	7	0
32.0	6	2
32.5	14	6
33.0	7	1
33.5	5	16
34.0	0	8
34.5	1	10
35.5	0	4
Total	48	47

Table 2. *April*
Temperature operative period 1 hr. Examined 24 hr. later

Temp. of water °C.	No. alive after 24 hr.	No. dead after 24 hr.
30.1	10	0
31.0	10	0
31.5	7	0
32.0	8	2
32.5	12	6
33.0	2	8
33.5	0	18
Total	49	34

Table 3. *Bufo bufo*

Air temp. °C.	R.H. %	Body temp. T ₁ °C.	Wet-bulb temp. T ₂ °C.	T ₁ - T ₂ °C.
30.9	24	20.7	17.1	+3.6
30.8	38	22.6	20.5	+2.1
29.8	50	24.4	22.0	+2.4
29.2	49	23.1	21.5	+1.6
27.5	27	17.5	16.0	+1.5
27.5	41	20.1	18.7	+1.4
27.6	82	26.5	24.9	+1.6
26.8	58	22.5	20.8	+1.7
24.1	26	15.1	13.2	+1.9
24.8	34	16.9	15.3	+1.6
23.7	26	14.5	13.2	+1.3
19.5	49	14.6	13.4	+1.2
21.4	86	19.8	19.7	+0.1

do survive, however, at this temperature. Fig. 1 exhibits rough mortality graphs for *B. bufo* and *R. temporaria* based on the data in Tables 1 and 2. They suggest a slightly greater resistance of *B. bufo* to thermal death, but the difference for 50% mortality is only of the order of 0.5°C.

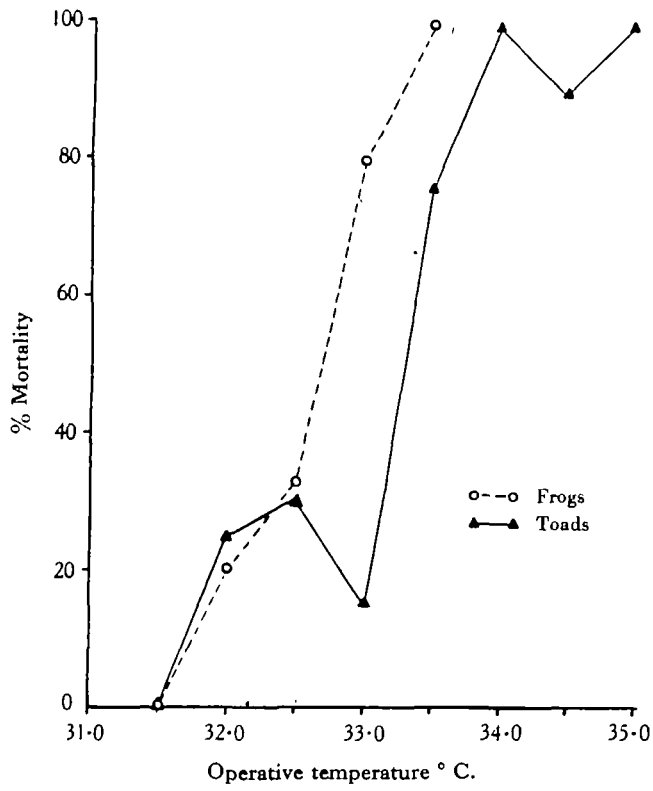


Fig. 1.

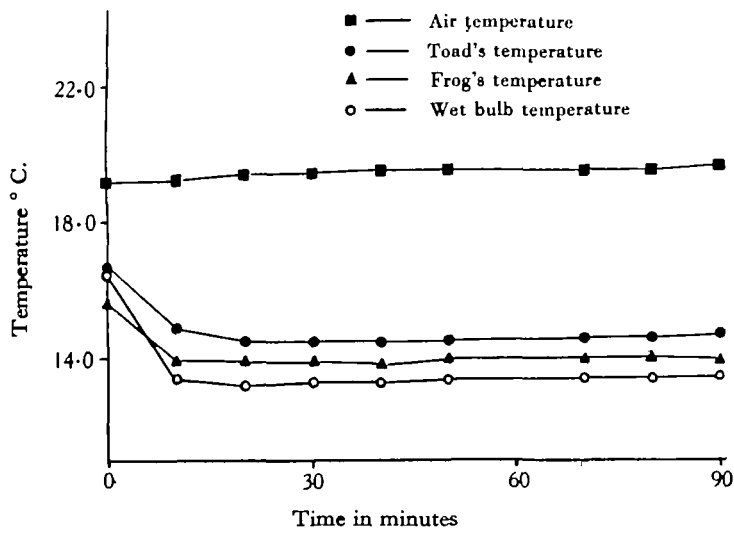


Fig. 2.

(b) *Body temperature.* Table 3 gives the results of a number of experiments at different air temperatures and humidities. At low humidities the body temperature of the toad, like that of the frog, is considerably below that of its surroundings, though generally well above that of the wet-bulb thermometer. In other experiments, recorded in Table 4,

In individual experiments the body temperature of the toad might be slightly in excess of that of the frog, or vice versa, but the mean value of Δt for all the experiments is zero. From the results of these experiments, therefore, it would seem that the toad loses water as freely as the frog, although there are individual variations in both species. This conclusion

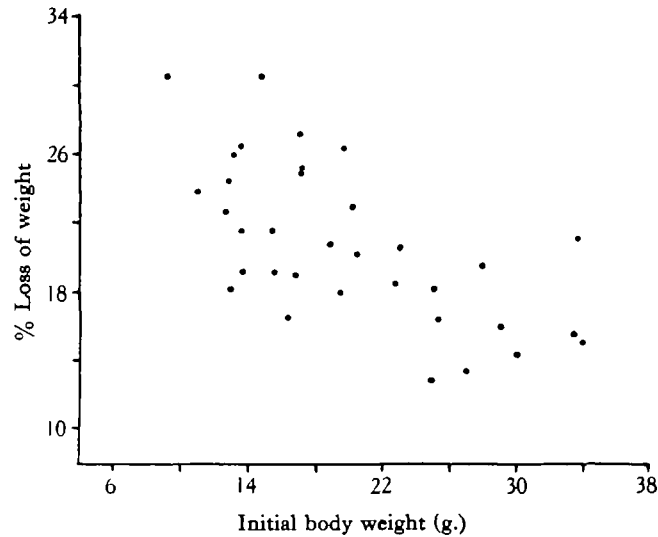


Fig. 3.

Table 4. Differences between rectal temperature of frogs and toads

$$\Delta t = t_{\text{toad}} - t_{\text{frog}}$$

Time (min.)	1	2	3	4	5	6	7
30	+0.2	0.0	-0.7	+0.9	-1.0	+0.6	+0.2
40	+0.3	0.0	-0.7	+0.8	-0.9	-0.1	+0.1
50	0.0	0.0	-0.5	+0.9	-0.8	-0.1	0.0
60	+0.3	0.0	—	+0.9	-0.6	-0.1	+0.1
70	+0.4	-0.3	-0.8	+0.7	-0.5	+0.7	0.0
80	+0.3	-0.3	-0.6	+0.8	-0.5	+0.8	-0.1
90	+0.2	-0.3	-0.6	+0.4	-0.4	-0.8	+0.6
Mean air temp. °C.	32.0 ± 0.1	27.4 ± 0.4	21.4 ± 0.2	24.6 ± 0.2	27.2 ± 0.2	26.6 ± 0.3	29.2 ± 0.3
Mean rectal temp. toad °C.	20.4 ± 0.4	26.4 ± 0.4	19.8 ± 0.2	16.7 ± 0.3	17.2 ± 0.3	22.3 ± 0.3	23.0 ± 0.2
Mean wet-bulb temp. °C.	17.0 ± 0.1	24.8 ± 0.2	19.7 ± 0.2	15.3 ± 0.0	15.8 ± 0.2	20.9 ± 0.1	21.6 ± 0.1
R.H. %	36	92	86	43	36	72	59

the body temperature of toads and frogs was measured at the same time, the animals being placed side by side in the experimental chamber. Care was taken to select animals of the same sex and of approximately the same weight. A direct comparison of the temperature response of frogs and toads to different environmental conditions is thus possible, and a typical experiment is shown in Fig. 2.

does not agree with the work of Hall & Root referred to above. In general their figures show that at 20°C. the depression of body temperature of the toad in relative humidities of 0-75% is only four-fifths that of the depression of the body temperature of the frog at a corresponding R.H. It is possible that these discrepancies may be due to the different species which were used by the American investigators. The

problem of the small intra species variations in body temperature was subject to further investigation in the case of the frog. A large number of animals was dehydrated under standard conditions, weights being recorded at regular intervals. Each frog was removed from water, and its bladder emptied by slight abdominal pressure. It was placed in a dry breffit jar covered with a light muslin top and kept at 25° C. and 75% R.H. in turbulent air. The scatter diagram of Fig. 3 exhibits initial body weight plotted against percentage loss of weight during a 48 hr. period. As would be expected, it reveals a decrease with respect to rate of loss of water as body weight increases, but the trend is subject to considerable individual variability. If we assume that the thermal capacity of frogs' tissue remains roughly constant this variability with reference to rates of water loss should correspond to individual discrepancies with respect to body temperature within the same species in identical external circumstances.

4. BODY TEMPERATURE OF REPTILES

(a) *Upper thermal death-point.* The upper thermal death-point of slow worms (*Anguis fragilis*) was determined by placing them in closed moist Petri dishes, 23 cm. in diameter and 5 cm. deep, in a thermostatically controlled incubator. The temperature inside the dish was measured by a mercury in glass thermometer inserted through the lid. A double-walled glass door fitted to the incubator facilitated observation of the animals during the course of an experiment. The air inside the dish was considered to be almost completely saturated with water vapour. At the beginning of an experiment four animals were removed from the tank in which they were kept and transferred rapidly to the Petri dish which was already at the desired temperature. At the end of 1 hr. the animals were transferred from the incubator to another closed Petri dish and kept at room temperature. Numbers alive and dead 24 hr. later are recorded in Table 5. From this it is clear that the temperature for 50% mortality in the laboratory stock is near 37.5° C. when the operative period is 1 hr. It is interesting to note that the state of reversible heat rigor such as affects the majority of frogs between 30 and 33° C. does not occur in slow worms. Almost invariably animals that fail to give eyelid response to mechanical stimulation at the end of the temperature operative period, also fail to recover during the next 24 hr.

(b) *Body temperature of the slow worms.* The body temperature of the slow worms under varying conditions of air temperature and humidity was determined by adopting the procedure already outlined in paragraph 2. The results of a series of experiments are recorded in Table 6. Twenty minutes after being placed in the experimental chamber the body temperature, as measured in the rectum, closely approximates that of the air. If the humidity of the air is low

the body temperature is slightly below that of the air. At higher humidities and air temperatures above 20° C., cooling by evaporation is apparently insufficient to carry away all the metabolic heat and the body temperature is raised above that of the air. Briefly, the results of these experiments indicate that over a wide range of air temperature and humidity the body temperature of the slow worm at no time differs from that of the air by more than 1° C. We have made no attempt to evaluate the effect of the temperature of the substratum on the body temperature of the slow worm. Under experimental conditions the temperature of the metal plate on which the

Table 5. October

Temperature operative period 1 hr. Examined 24 hr. later

Temp. of air ° C.	No. alive after 24 hr.	No. dead after 24 hr.
35.0	4	0
35.5	4	0
36.5	4	0
37.0	5	7
38.0	1	7
Total	18	14

Table 6. *Anguis fragilis*

$$\Delta t = t_{\text{air}} - t_{\text{rectum}} \text{ } ^\circ\text{C.}$$

Air temp.	R.H. %	Δt after			Mean value of Δt ° C.
		20 min.	30 min.	40 min.	
30.7	19	+0.5	+0.3	+0.2	+0.33
29.7	38	0.0	+0.2	+0.7	+0.30
28.4	64	-0.1	-0.4	-0.3	-0.27
26.8	18	+0.3	+0.3	+0.1	+0.23
23.1	71	-0.6	-0.6	-0.7	-0.63
21.6	27	+0.2	0.0	0.0	+0.07
17.3	45	+0.2	+0.2	+0.2	+0.20
17.3	53	+0.3	+0.4	+0.2	+0.30
16.8	98	+0.2	0.0	-0.2	0.00
5.6	85	-0.3	+0.2	+0.2	+0.03
4.0	96	-0.2	0.0	+0.1	-0.03

animal crawled was identical with that of the air. In the field such an agreement between air and ground temperatures is exceptional, and it is likely that the ground temperature is an important circumstance contributory to the body temperature of limbless tetrapods such as the slow worm and *Ophidia*.

(c) *Body temperature of the alligators.* Two young alligators (*Alligator mississippiensis*) were used for further investigations of the body temperature of reptiles. They were almost of identical size, being 1.08 and 1.10 m. long, respectively weighing 4.77 and 4.33 kg. We have not deemed it necessary to distinguish between them in the record of results.

Table 7 records mean temperature measured under the conditions described in § 2 above. The air temperature and humidity were kept fairly constant during the course of an experiment. To enable body temperature to reach steady value, several hours after placing an animal in the experimental chamber

out to investigate the way in which body temperature lags behind that of the air when it is fluctuating. Fig. 4 is a typical example of such an experiment. The animal was in the experimental chamber over a period of 24 hr. and the air temperature was suddenly changed on two occasions. It reached a maximum

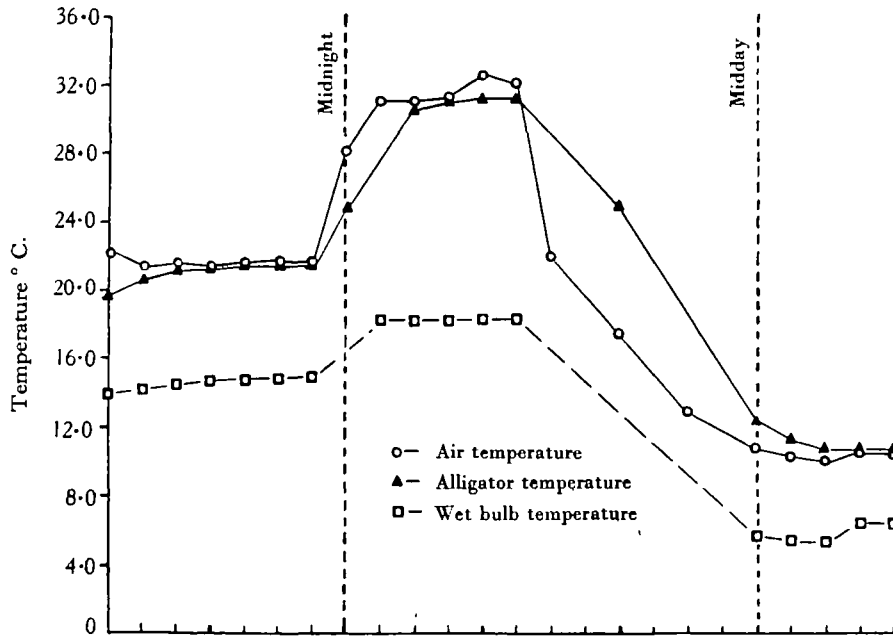


Fig. 4.

Table 7

Exp. no.	Mean air temp. T_1 °C.	R.H. %	Mean alligator temp. T_2 °C.	Range air temp.	Range body temp.	Duration of exp. hr.	$T_2 - T_1$ °C.
1	29.2	33	29.4	±0.6	±0.1	3	+0.2
2	27.8	59	27.5	±0.4	±0.3	4	-0.3
3	25.6	43	26.2	±1.0	±1.0	6	+0.6
4	21.6	78	21.5	±0.3	±0.1	5	+0.1
5	21.1	50	21.1	±0.2	±0.1	4	0.0
6	15.3	83	15.1	±0.1	±0.2	4	-0.2
7	10.3	60	10.7	±0.4	±0.1	4	+0.4
8	7.2	52	7.4	±0.1	±0.1	5	+0.2

were allowed to elapse before taking readings. From the magnitude of the difference between mean body temperature and mean air temperature, it would seem that the alligator's temperature does not differ significantly from that of the surrounding air over long periods of time, when conditions are maintained relatively stable. Other experiments were carried

value of 32.5° C. and a minimum value of 10.0° C. When the temperature was raised from 22 to 31° C. the body temperature was still 0.5° C. below the air temperature after 3 hr., and when the air temperature was reduced from 32 to 10° C. the alligator still had a body temperature of 0.6° C. higher than the air temperature 9 hr. later.

5. DISCUSSION

Adaptation of vertebrates to a land habitat necessarily involved a complex set of physiological and anatomical changes which together enabled them to live successfully away from the relatively stable environment of their aquatic ancestors. Structural innovations permitting locomotion on land were of little use without behaviour patterns to ensure the fertilization of eggs in a watery medium, and the advantages of either an impermeable integument or of a calcareous shell during the period of development could not be fully exploited until the tissues were themselves adapted to withstand the effects of high temperature. This adaptation was a necessary precursor of the development of homoeothermy in diurnally active animals. Extensive desiccation would have been the price of stabilizing body temperature at a level lower than that of mammals or birds, and this would have militated against maintenance of stable osmotic pressure of the blood and tissues. Without such osmotic stability, the regulation of a high grade of metabolic and nervous activity such as exists in the mammals and birds would be difficult to conceive.

That such a complex series of changes has taken place is self-evident, but the steps by which they were effected are still obscure from a physiological viewpoint. As has been pointed out previously, Gray (1928) drew attention to the necessity for developing an impermeable integument both for the egg and the adult before extensive conquest of land by vertebrates could proceed. Subsequently, Hall & Root (1930) subjected to experimental enquiry the consequences of the possession of an impermeable integument *vis-à-vis* body temperature. In broad outline, the present investigation supports the main conclusions of Hall & Root, and in particular the thesis that the increase in resistance to desiccation involved a concomitant increase in, if not a pre-development of, resistance to thermal death at high temperatures. It was, perhaps, unfortunate that circumstances dictated the use of a reptilian species which is by habit cryptozoic. Nevertheless, the thermal death-point of the slow worm is significantly above that of the amphibian species investigated. Although reliable information on the upper thermal death-point of vertebrates is difficult to obtain, the work of other investigators (Cole, 1943) gives us reason to believe that the thermal death-point of typically basking reptiles is considerably above that of the slow worm, and in some species may be as high as 45° C.

In the development of temperature regulatory mechanisms the size of an animal must itself have played a significant part. It has been suggested that the giant reptiles of the Jurassic and Cretaceous may have had a body temperature considerably above the temperature of the air, because of their large bulk and relatively small surface area. This supposition

has been discussed by Gunn (1942) who concludes that it is unlikely on the basis of data recorded by Benedict. He suggests that body temperature would follow air temperature sluggishly and fluctuate around a level intermediate between the maximum and minimum experienced during the course of 24 hr. Our own observations on the alligator add weight to this conclusion. The slow worm reaches equilibrium within half an hour, even when temperature changes are sudden and drastic. The body temperature of young alligators of about 4 kg., i.e. one hundred times as heavy as the largest slow worm in our experiments, required about 10 hr. to drop 20° C. when the air temperature was reduced suddenly. The largest extinct reptiles were many hundred times as bulky as our alligators, and it would therefore seem reasonable to suppose that body temperature would fluctuate over a relatively small range. Activity and success could only be assured, however, so long as the mean external temperature remained at a level high enough to permit a rapid movement and the efficient performance of all bodily functions. As the mean external temperature dropped, smaller forms able to take advantage of short periods of high temperature by day and to remain quiescent by night or hibernate during winter would have a better chance of survival. One further consequence of the possession of large body size in reptilian stock would seem to be that a considerable elevation of the metabolic rate would impose upon the animal the need for a cooling mechanism involving copious water loss during periods of high *internal* temperature resulting from previous exposure to high external temperatures.

6. SUMMARY

1. The upper thermal death-point of the slow worm (*Anguis fragilis*) is significantly above that of either the toad (*Bufo bufo*) or the frog (*Rana temporaria*). That of the toad differs only slightly from that of the frog.

2. In response to external variation with respect to temperature and humidity, the body temperature of the toad behaves in a manner similar to that of the frog, being considerably below that of the air at low humidities, though appreciably above that of the wet-bulb thermometer. It is concluded that the toad loses water as freely as the frog.

3. The body temperature of the slow worm and that of the alligator (*A. mississippiensis*) differs only slightly from that of the air under all conditions of *sustained* air temperature and humidity.

4. The body temperature reaction of the alligator is more sluggish than that of the slow worm, presumably owing to the higher thermal capacity of the animal.

5. The significance of these results in relation to the evolution of the terrestrial vertebrates is

discussed. It is suggested that homoeothermic stocks could arise only from reptiles of relatively small size, and that large body size would militate against survival during periods of low *mean* external temperature.

We wish to acknowledge financial assistance in a grant made to the Department by the Rockefeller Foundation and an emolument from the Halley Stuart Trust during the course of this work.

REFERENCES

- COLE, LAMONT C. (1943). *Ecology*, **24**, 94.
GRAY, J. (1928). *Brit. J. Exp. Biol.* **6**, 26.
GUNN, D. L. (1942). *Biol. Rev.* **17**, 293.
HALL, F. G. & ROOT, R. W. (1930). *Biol. Bull. Woods Hole*, **58**, 52.
HOGBEN, L. T. & KIRK, R. L. (1944*a*). *Proc. Roy. Soc. B*, **132**, 68.
HOGBEN, L. T. & KIRK, R. L. (1944*b*). *Proc. Roy. Soc. B*, **132**, 239.