the agar in the dark. In addition, it was possible, by means of adaptation, to manipulate the responses so as to produce negative or positive potentials. An example of such a manipulation is pictured in Fig. 2. Starting with the response of record 2, the eye was illuminated with light at 600 nm, used because eyes at this stage are very sensitive to these longer wavelengths; during this steady illumination the potentials elicited by flashes of white light superimposed on the orange adapting field were observed. Over a period of 10 minutes of light adaptation, evidence of a growing positivity was obtained, the record secured at the 10th minute being shown as record 3 (Fig. 2). The adapting field was then turned off; 1 minute later the response to the white light flash had become quite large and purely negative (record 4). The eye was then allowed to remain in the dark, during which time it gave responses which changed again to a form (records 5 and 6) consisting of a positive wave following an initial negative deflection. We do not understand the basis for this complex lability at this stage. Light and dark adaptation appear to be involved, but other factors, as yet undetected, also may be concerned. All that we wish to point out at this time is that this period of lability is unique and that this behavior may be employed to differentiate the eyes prior to period III and following period III.

Period IV: after the 17th day. In larvae older than *about* 17 days (this time has not been critically examined) the ERG showed all the characteristics of the ERG of the adult larva. No lability like that associated with period III was noted, and the records showed positivity from the first moment of recording and the positivity increased with dark adaptation in a typical manner. This sequence of change covered by these four periods was recorded with striking regularity in all four batches of embryos. In addition, it was also duplicated in a study of embryos of the bullfrog (*Rana catesbiana*).

The visual cells of the frog retina develop, typically as in other animals, after the neural layers have formed (*7*). Nilsson (*8*) observed the first appearance of the outer segment discs in *Rana pipiens* to be at the age of 5 days and 20 hours. The first electrical activity which was recorded was therefore about 1 day later than this. This early electrical activity was characteristically different from that elicited from the fully developed retina. This is in agreement with the results of some previous workers (*1–3*) but not with those of others (*4–6, 9*). The disagreement is only an apparent one in some cases. Kennedy (*9*), for example, employed tadpoles of *Rana pipiens* which were collected in the field and which were not staged. In a letter to us, Kennedy explained that the animals "were as near to the point of metamorphosis as possible, simply for size reasons." Thus his animals were well outside what we have here called period III. With respect to Muntz's results (*6*), we have a letter from him which states that the spawn was released in the laboratory on 20 March 1963 and that it was 2 April before an ERG was recorded. This would place his embryos from which he first recorded an ERG at the 13-day-old stage, well into the period of *b*-wave development. There is no difference, therefore, between our results and those obtained by Muntz. We have no explanation for the failure of Müller-Limmroth and Andrée (*5*) to record a unique ERG in the early stages of development. Their animals, when first tested, appear to have been young enough for the early, negative responses to have shown themselves. We conclude, therefore, that our results are in essential agreement with those who assert the existence of a change in form of the ERG during development of the retina.

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Cutaneous Water Loss in Reptiles

Abstract. *Cutaneous and respiratory evaporation were compared in five species of reptiles at 23°C. There seemed to be a clear correlation between water loss and aridity of the animal's habitat, total evaporation from the desert lizard* Sauromalus obesus *being about 5 percent of that from the crocodilian* Caiman sclerops. *Cutaneous evaporation was the major avenue of water loss in all animals examined. This is contrary to the common belief that reptilian skin is practically impermeable to water.*

Reptiles phylogenetically represent the first vertebrates to become truly terrestrial. As opposed to the Amphibia they are considered to have an integument quite impermeable to water, and it has often been emphasized that this was important in their adaptation to terrestrial conditions. Recently, however, we have shown that the crocodilian *Caiman sclerops* loses water by evaporation at a surprisingly high rate, one-half to one-third as fast as amphibians (*1*). It seems probable that this reflects a greater diversity in permeability of the integument in different reptile groups than was formerly believed.

In our study we have compared cutaneous and respiratory water loss in two species of turtles, two species of lizards, and one crocodilian, which represent three different evolutionary lines. We found large differences in both cutaneous and respiratory water losses which seem correlated with the environments in which these animals usually live.

Evaporation was estimated from weight loss of the animal with a correction for weight loss due to metabolic loss of carbon. Urine and fecal losses were either prevented by taping or measured by cannulating the cloaca. Weight loss from the head (principally respiratory tract) and from the rest of the body (skin) was measured separately by keeping the body in a polyethylene bag (thickness, 0.1 mm), containing a desiccant to prevent possible diffusion loss of water, which was sealed around the neck with adhesive tape. Water loss from the head was obtained by placing the animal in a plastic chamber, passing dry air slowly over the animal, and then determining the weight loss. By subtracting the loss

Table 1. Respiratory and cutaneous water loss in various reptiles in dry air at 23°C. Results are expressed as means ± S.E. Numbers of each species tested in parentheses.

Body wt. (g)	Total wt. loss (mg cm^{-2} day^{-1})	Oxygen consumption (ml g^{-1} day^{-1})	Respiratory			Cutaneous	
			Wt. loss (mg g^{-1} day^{-1})	Wt. loss (mg/ml O_2)	Water loss (mg/ml O_2)	Water loss (mg cm^{-2} day^{-1})	Water loss (% of total)
	(a)	(b)	(c)	(d)	(e)	(f)	(g)
			Caiman sclerops (8)				
124	37.7 ± 2.11	1.8 ± 0.05	9.6 ± 1.2	5.3 ± 0.91	4.9	32.9 ± 2.45*	87 ± 2.1
			Pseudemys scripta (6)				
600	15.8 ± 1.70	0.9 ± 0.12	4.3 ± 0.48	4.7 ± 0.88	4.2	12.2 ± 1.44	78 ± 2.7
			Terrapene carolina (6)				
305	7.2 ± 0.31	0.6 ± 0.05	2.6 ± 0.29	4.6 ± 0.53	4.2	5.3 ± 0.41	76 ± 3.4
			Iguana iguana (8)				
124	6.7 ± 0.41	2.6 ± 0.36	3.4 ± 0.48	1.3 ± 0.21	0.9	4.8 ± 0.50	72 ± 4.3
			Sauromalus obesus (6)				
134	2.0 ± 0.168	1.2 ± 0.96	1.1 ± 0.05	0.9 ± 0.08	0.5	1.3 ± 0.10	66 ± 2.0

* The difference in cutaneous water loss between *C. sclerops* and *P. scripta* is $P < .001$, and between *P. scripta* and *T. carolina*, $P < .001$; but the difference between *T. carolina* and *I. iguana* is not significant; between *I. iguana* and *S. obesus* the difference is $P < .001$.

Table 2. Evaporative water loss from *Sauromalus obesus* in dry air at 23° and 40°C. Numbers of animals tested are in parentheses. Results are expressed as means ± S.E.

Temperature	Water loss			Oxygen consumption (ml g^{-1} day^{-1})
	Total (mg cm^{-2} day^{-1})	Respiratory (mg g^{-1} day^{-1})	Skin (mg cm^{-2} day^{-1})	
23°C	1.7 ± 0.17(6)	0.6 ± 0.08(6)	1.3 ± 0.10(6)	1.2 ± 0.96(6)
40°C	7.8 ± .7(9)	8.1 ± 2.5(5)	3.4 ± .55(5)	5.5 ± 2.6(7)

of carbon (CO_2 estimated from the metabolic rate) and the water loss from the skin of the head (using the mean rate of cutaneous water loss determined separately), respiratory water loss was obtained.

Cutaneous water loss was determined as the difference between total weight loss and respiratory water loss as obtained in alternate experiments on the same animal. The surface area of the animals was calculated from the equation: area (cm^2) = 10 body weight (grams)$^{\frac{2}{3}}$, as suggested by Benedict (*2*). Oxygen consumption was determined in an open system where air was passed over the animals at a constant rate and change in partial pressure of oxygen was determined with a Beckman paramagnetic oxygen analyzer. After an equilibration period of 1 to 3 hours, values were recorded at 15-minute intervals for 1 hour, and the mean value was used.

Total water loss varied considerably in the different reptiles. For example, water loss per surface area was 19 times as high in the crocodilian *Caiman sclerops* as in the desert lizard *Sauromalus obesus* (Table 1, column a). The figures represent total weight loss (excluding excreta), but subtraction of metabolic carbon (about 0.5 percent of the total in *Caiman* and about 10 percent in *Sauromalus*) would not materially change the relation. In all species examined, water loss shows a conspicuous correlation with habitat, decreasing drastically with increasing aridity.

The importance of water loss to an animal is perhaps more evident if we consider the loss that is sustained over a 24-hour period. Calculated as the percentage of body weight evaporated per 24 hours, the figures (corrected for metabolic carbon) would be: *Caiman* 11.5, *Pseudemys* 2.0, *Terrapene* 0.9, *Iguana* 0.8, and *Sauromalus* 0.3 percent. While the *Caiman* lost one-tenth of its body weight by evaporation in a day, it would take *Sauromalus* a month to reach the same degree of dehydration.

Respiratory weight loss also shows a trend correlated with the habitat of the animal (Table 1, column c). However, it seems more meaningful to relate respiratory water loss to oxygen consumption because evaporation from the lungs should increase with increasing oxygen consumption (Table 1, columns d and e). It is apparent that the crocodile and the two turtles sustain a respiratory water loss of about 5 mg of H_2O per milliliter of O_2 in contrast with less than 1 mg of H_2O per milliliter of O_2 in the two lizards. The difference between any of the former three and the last two is highly significant ($P < .001$).

Respiratory water loss of reptiles (Table 1, column e) is surprisingly high as compared to that in mammals. Only in the two lizards is it similar to the figure for man, whose respiratory evaporation is about 0.85 mg of H_2O per milliliter of O_2, and to the range of total evaporation in a variety of small nonsweating rodents where it may be as low as 0.5 mg of H_2O per milliliter of O_2 (*3*). The figures in Table 1 were obtained at a temperature of 23°C. We have determined the evaporation at higher temperatures only in *Sauromalus*, where at 40°C it was 1.35 mg of H_2O per milliliter of O_2, still close to the mammalian range.

Cutaneous water loss (Table 1, column f) also decreases successively with aridity of the habitat. In the crocodilian *Caiman sclerops*, cutaneous water loss was of an order of magnitude about one-third of the water loss from the skin of amphibians (*4, 5*). This is surprising in view of the common belief that the skin of reptiles is essentially impermeable to water. Cutaneous water loss from the aquatic turtle *Pseudemys scripta* is considerably less, but still 10 times as high as in the desert lizard *Sauromalus obesus*. In the two terrestrial forest dwellers, the box turtle *Terrapene carolina* and the tropical lizard *Iguana iguana*, cutaneous water loss is identical and about four times as high as in the desert lizard.

The role of cutaneous water loss in total evaporation from these reptiles is given in the last column of Table 1. It is astonishing to find that in all the reptiles that we examined cutaneous evaporation was two-thirds or more of the total evaporation. At a higher temperature respiratory evaporation can be expected to increase because of increased metabolism and because warm air can contain more water vapor. Cutaneous evaporation should also increase and was found to do so in about the same proportion as the increase in vapor pressure. The relative importance of the skin in water loss should therefore diminish. Table 2 shows a 4.6-fold increase in total evaporation in *Sauromalus* between 23° and 40°C, composed of a 10-fold increase in respiratory evaporation and a 2.6-fold increase in cutaneous evaporation. This constitutes a decrease in the contribution of the skin to total evaporation

from about 60 to about 40 percent. These findings show that, even in the desert lizard *Sauromalus*, cutaneous evaporation is a major avenue for water loss. This is contrary to the common belief that reptilian skin is essentially impermeable to water.

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Residues of DDT in Brains and Bodies of Birds That Died on Dosage and in Survivors

Abstract. *Residues of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)-ethane (DDT) and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethane (DDD) in brains of cowbirds* (Molothrus ater) *killed by dietary dosage of DDT were similar in birds that died after various lengths of time on dosage and in birds that died of delayed effects after as much as 40 days on clean food. Residues of DDT and DDD, but not of 1,1-dichloro-2,2-bis-(*p*-chlorophenyl)-ethylene (DDE), were much lower in survivors 112 days after dosage. The relative importance of DDT and DDD in brains could not be determined, but DDE appeared not to be critical. Residues in brains of cowbirds were similar to those reported for robins, sparrows, eagles, and white rats. Residues in livers and carcass remainders (with the possible exception of DDD in the liver) appeared unsuitable for diagnosing the cause of death.*

Probabilities that deaths of birds in the field are due to pesticides are difficult to establish because neither times of exposure nor dosages are known. For residues to be most useful in diagnosing the cause of death, levels in dead animals should be similar regardless of either dosage or time on dosage before death. This has been true of residues of DDT found in brains of sparrows (*Passer domesticus*) studied experimentally (*1*) and in laboratory rats (*Rattus norvegicus*) (*2, 3*). Correlative evidence has appeared in data on residues in robins (*Turdus migratorius*) collected tremoring or dead after DDT treatments for the control of Dutch elm disease (*1, 4*). However, little attention has been given to the relations between the metabolites involved, to the residues present in acute versus delayed mortality, or to comparisons between residues in the brain and in other portions of the body.

These relations are shown here for a group of experimental cowbirds that had been fed DDT in their diet. Comparisons are made of residues in birds dying at various times during dietary dosage, in those dying after dosage, and in the survivors. General validity of the conclusions is discussed by comparing various other species for which data could be obtained.

In our study, 27 cowbirds were fed a diet containing 500 parts per million (ppm) of *p,p'*-DDT. The DDT was dissolved in cottonseed oil and mixed into the diet of turkey-starter crumbles. Thirteen birds in one cage were fed toxicant until all had died, a period of 12 days. In another cage, 14 birds were fed toxicant for 8 days, at which time seven had died. The seven remaining birds were given clean food for the duration of the experiment. Four of the seven died after 2, 9, 40, or 93 days on clean diet; three survived for 112 days and were killed. Severe tremoring, typical of DDT poisoning, was observed in four of the birds that died while on the toxic diet and in the one bird that was dead after 9 days on clean food. Deaths of the other birds were not observed.

Autopsy of all birds showed conditions common to cowbirds succumbing to DDT poisoning: Fat was essentially gone from the visible storage sites; there was no muscular emaciation (pectorals and other muscles were full and firm); and the gall bladder was full.

Brains, livers, and remainders (remaining parts of the specimen exclusive of the digestive tract, which was dissected out and discarded) of 17 male cowbirds were analyzed (*5*) for DDT residues—11 individually, the remaining 6 as a pool.

DDT and DDD levels in the brains of dead cowbirds showed no time-related trends. They were similar in birds that died after varying times on dosage and in those that died after 2 to 40 days on clean food. DDT plus DDD averaged 66 ± 6 ppm wet weight, with a range of 35 to 99 ppm. The effects of DDT cannot be separated from those of DDD, for brain levels of the two were similar and both were always present. Brains of birds that died 9 and 40 days after dosage contained more DDE than the brains of birds that died during dosage did. Brains of survivors, killed after 112 days, contained more DDE than most birds that died on dosage. Hence, DDE appears not to be critical in this series. Brains of birds that died contained less DDE than either DDD or DDT, a relation that was reversed in survivors. It is clear that more and more DDE was being produced from residues elsewhere in the body.

Quantities of DDT residues in brains of cowbirds were remarkably similar to those reported for a variety of other species, when expressed comparably and with allowance for differences in chemical methodology. In Table 1, data for the cowbirds in this study, robins (*1, 4, 6*), sparrows (*1*), eagles (*Haliaetus leucocephalus*) (*1*), white rats (*2, 3*) are compared. It appears that similar brain levels of DDT and DDD combined may be associated with DDT-induced mortality over a wide range of species, mammals as well as birds.

Despite these similarities, the residue levels in the brains of individuals that died from DDT poisoning varied widely (Table 1). Thus we cannot set a single level that is positively diagnostic of death. It is of practical value, however, to recognize the range within which lethal levels fall. When a level falls within this range, it seems entirely fair to say that the animal was at least seriously endangered by the poisoning. The probability that an animal was killed by DDT obviously is far greater at high residue levels than at low ones.

The critical question is how low a level may be taken to represent serious danger and possible death from DDT. From all available data, including