aspect of alcoholism. Centrally administered TIQ alters alcohol withdrawal seizures in mice (*21*), which may indicate that TIQ's have a role in the dependence syndrome. Also, results have suggested that amine condensation products may sustain abnormal alcohol-drinking behavior (*8*). A third possible route of involvement, suggested by rat studies in alcohol preference (*22*) and by biochemical and histochemical results with an adrenaline-derived TIQ (*23*), is that TIQ's induce relatively permanent changes in the nervous system. These possibilities take on added meaning in view of our results.

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13. Measurement of acetaldehyde in human blood by headspace gas-chromatography techniques has been complicated by artifactual generation of acetaldehyde during deproteination and incubation [C. Eriksson, H. Sippel, O. Forsander, *Anal. Biochem.* **80**, 116 (1977); A. Stowell, R. Greenway, R. Batt, *Biochem. Med.* **18**, 392 (1977)]. Control studies showed that the artifact could be minimized by using low incubation temperatures and thiourea [W. Nijm, G. Borge, T. Origitano, G. Teas, C. Goldfarb, M. Collins, *Res. Commun. Chem. Pathol. Pharmacol.* **20**, 187 (1978)]. The blood acetaldehyde values we obtained are in approximate agreement with some studies of uncontrolled drinking in alcoholics but several times higher than those obtained in recent studies involving controlled alcohol administration.
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Temperature-Dependent Sex Determination in Turtles

Abstract. *The sex of hatchling map turtles is determined by incubation temperature of eggs in the laboratory as well as in nature. Temperature controls sex differentiation rather than causing a differential mortality of sexes. Temperature has no effect on sex determination in a soft-shelled turtle.*

Genotypic sex determination is nearly ubiquitous in tetrapod vertebrates. In many species genotypic control is manifested in morphologically distinct sex chromosomes (mammals, birds, some reptiles), but genotypic control is also known in species that lack detectable sex chromosomes (amphibians) (*1*). There are some reptiles, however, in which genotypic control of sex determination has not been demonstrated and in which incubation temperature of the eggs affects the sex ratio of hatchlings in the laboratory (four turtles, one lizard) (*2, 3*). Possibly, temperature is the sex determining agent in these species (*3*), but evidence from incubation under natural conditions is lacking. We present data obtained in the laboratory and field which indicate that temperature is a natural determinant of sex in map turtles but not in a soft-shelled turtle.

Previous laboratory studies of turtles have shown that constant incubation temperatures of 31°C and above produce female hatchlings, cooler temperatures (24° to 27°C) produce males (*2, 3*), and, in the snapping turtle (*Chelydra*), even cooler temperatures (20°C) again produce females (*3*). It is likely in *Chelydra* that temperature is controlling sex differentiation rather than causing differential mortality of the sexes because more than 80 percent of the eggs hatch (*3*), but data for the other species do not warrant such a conclusion. There has been little work on sex determination in turtles under natural conditions. Results from artificial incubation at constant temperatures do not necessarily apply to field situations because nest temperatures fluctuate in some species (*4, 5*). A laboratory study in which eggs of *Emys* (European pond turtle) were incubated at fluctuating temperatures in fact produced males and females (*6*). Field incubation of *Emys* eggs produced a male-biased sex ratio in accord with low soil temperatures (*5*), but the study included partial laboratory incubation at low temperatures, and the eggs were buried at sites not necessarily representing the parental population. Thus, uncertainties remain in interpreting the existing studies.

In 1978 we initiated a comparative study to survey several species of turtles for temperature effects on sex ratios of hatchlings, to observe whether the mechanism was due to sex determination or differential mortality, and to contrast results of incubation in field and laboratory settings. We performed three types of incubation experiments: experiment 1, constant temperatures; experiment 2, controlled, fluctuating temperatures; and experiment 3, natural, field, incubation. Each experiment consisted of two temperature regimes, one warm and one

0036-8075/79/1207-1186$00.50/0

cool, and eggs from each clutch chosen for the experiment were divided equally between the two. In experiment 1 eggs were incubated in the laboratory at 25° or 30.5°C. In experiment 2 eggs were incubated in the laboratory at daily temperature cycles ranging either from 20° to 30°C or from 23° to 33°C. The cycle consisted of 4 hours of incubation at each extreme and a linear change in temperature over 8 hours between the extremes (daily means of 25°C, 28°C). In experiment 3 eggs were buried at one of two sites on the nesting beaches, the sites differing in their exposure to the sun. In the site exposed to the sun, nest temperatures often exceeded 30°C (up to 37°C) for part of the day, but nest temperatures rarely reached 30°C in the shaded site.

Five species of turtles were studied from the Mississippi River near La Crosse, Wisconsin: *Graptemys geographica, G. ouachitensis* (*7*), *G. pseudogeographica, Chrysemys picta* (map and painted turtles, family Emydidae), and *Trionyx spiniferus* (soft-shelled turtle, family Trionychidae), although not all species were studied in every experiment (Table 1). Morphologically recognizable sex chromosomes are unknown in both families (*8*), and temperature is suspected of influencing sex determination in an emydid (*2*). Eggs were obtained either from gravid females (*9*) or from fresh nests, and experimental incubation was initiated within 48 hours. In the field experiment (experiment 3), artificial nests were constructed by hand to approximate natural nests. There is no postlaying parental care in turtles, and nests are merely cavities that have been excavated in the sand (10 to 20 cm deep), filled with 6 to 20 eggs, and covered with sand by the mother. Each half clutch was surrounded with a nylon net bag to contain the hatchlings and was buried with another half clutch to approximate the usual egg number per nest cavity. The artificial nests within each site were placed at intervals of 50 cm to ensure uniform exposure among all nests. The sites chosen for the artificial nests coincided with nests constructed by turtles.

Sex was diagnosed by inspection of gonads and accessory structures when viewed under a dissecting microscope (*10*). Nearly all individuals were analyzed at hatching or shortly after, and approximately half in each experiment were maintained alive for 1 to 3 months prior to dissection, so that the progress of gonadal differentiation could be observed.

In *Trionyx*, the sex ratio approximated 1:1 independently of incubation temperature (Table 1). In all experiments with the emydids, however, the progeny from the warm temperature are entirely or mostly female, while the progeny from the cool temperature are entirely male, as in previous studies with constant temperature incubation (*2, 3*). These data are consistent with a hypothesis of environmental sex determination but are not consistent with hypotheses of (i) predetermination of sex with differential mortality or (ii) preferential assortment of sex chromosomes in forming the zygote. With respect to (i), under the null hypothesis that sex is determined prior to the experiment (for example, by genotype), a significant difference in sex ratio between the warm and cool temperatures of an experiment must be accounted for by the unknowns (embryos failing to develop to near hatching). Yet, even if all unknowns are assumed to be of the opposite sex as the hatchlings at the same temperature, the null hypothesis is highly improbable in all emydids (experiments 1 and 2, Fisher's exact test). The null hypothesis is improbable in experiment 3 only for *G. ouachitensis* and *G. pseudogeographica*, and is highly improbable for the three species combined. With respect to (ii), the stages in which temperature affects sex determination in *Graptemys* are those in the middle third of development [revealed in a series of temperature-shift experiments as in (*3*)]. Therefore no peculiar assortment of genes at conception or even during cleavage can account for the sex ratio biases. The most plausible hypothesis for the sex ratio biases is that incubation temperature influences sex differentiation.

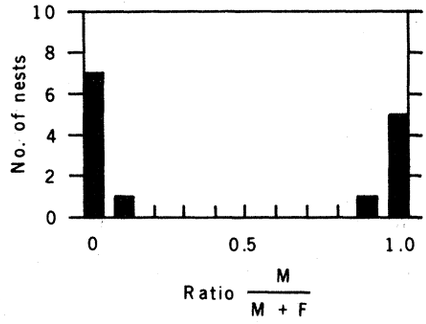

Fig. 1. Sex ratios in 14 natural nests of *Graptemys*. Clutch size is greater than or equal to 8. The progeny from a single nest tends to be of one sex, but some nests produce males and others produce females. *M*, number of males in the clutch; *F*, number of females.

A small sample of hatchlings was collected from natural nests (Fig. 1). The sex ratios in these are consistent with the experimental observations. There is a bimodal distribution of sex ratios in nests of *Graptemys*. Hatchlings from shaded or partially shaded nests were nearly always male. The single natural nest of *Trionyx* hatchlings examined contained seven males and six females.

We have yet to demonstrate that these individuals will maintain their sex constitution until maturity and be fertile. The lengthy immature period in these turtles (4 to 10 years) precludes an immediate answer to this question. Some observations indirectly suggest that sex reversal is unlikely, however. (i) Intersexes are uncommon among hatchlings. In experiments producing both sexes, there is usually a clear-cut dichotomy among males and females even though both ex-

Table 1. Sex ratios of hatchling turtles. The question mark indicates sex unknown: infertile, or dead at early stages.

Sex	Experiment 1		Experiment 2		Experiment 3	
	25°C	30.5°C	20° to 30°C	23° to 33°C	Shade (*13*)	Sun
			Graptemys ouachitensis			
Male	210	0	73	0	100	4
Female	0	211	0	65	0	123
?	23	26	38	44	101	74
			Graptemys pseudogeographica			
Male	173	4	43	0	35	1
Female	0	147	0	43	0	19
?	49	81	20	24	10	25
			Graptemys geographica			
Male	98	0			37	0
Female	0	88			0	15
?	24	31			12	36
			Chrysemys picta			
Male	81	0				
Female	0	81				
?	21	20				
			Trionyx spiniferus			
Male	33	27				
Female	34	24				
?	16	35				

perienced the same temperature. (ii) Temperatures late in development have no influence on sex determination (on the basis of our temperature-shift experiments), and gonadal differentiation after hatching progresses in the direction expected from incubation temperature. These observations indicate that one of two clear-cut developmental pathways is undertaken in the embryo, and that the pathway is adhered to at least shortly after hatching. In map turtles raised 7 years from hatching, secondary sexual characteristics have remained constant from their inception at age 2 years (*11*). Histological studies on other turtles (*12*) showed no incidence of sex reversal or dysfunction in individuals of various ages. Therefore, we think it unlikely that infertility or changes in sex phenotype are common occurrences in these turtles, but this remains to be demonstrated conclusively.

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7. Here we consider *G. ouachitensis* as a valid species (R. C. Vogt, in preparation), although it is recognized by some as a subspecies of *G. pseudogeographica*. Our conclusions do not change if data for these two taxa are lumped.
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Target Cells for 1,25-Dihydroxyvitamin D_3 in Intestinal Tract, Stomach, Kidney, Skin, Pituitary, and Parathyroid

Abstract. *After mature rats that had been fed on a vitamin D_3–deficient diet were injected with tritium-labeled 1,25-dihydroxyvitamin D_3, radioactivity became concentrated in nuclei of luminal and cryptal epithelium of the duodenum, jejunum, ileum, and colon; in nuclei of the epithelium of kidney distal tubules including the macula densa, and in podocytes of glomeruli; in nuclei of the epidermis including outer hair-shafts and sebaceous glands; and in nuclei of certain cells of the stomach, anterior and posterior pituitary, and parathyroid. These results reveal cell types that contain receptors for 1,25-dihydroxyvitamin D_3 or metabolites of this compound both in known or hypothesized target tissues and in tissues that were previously unknown to participate in vitamin D_3 metabolism.*

Target tissues for 1,25-dihydroxyvitamin D_3[1,25$(OH)_2D_3$] have been demonstrated in intestine, kidney, and bone by means of radioassay and biochemical techniques (*1*). However, little information is available on the morphological identification of target cells for this hormone. In autoradiographic experiments with chick duodenum, radioactivity was found in the region of nuclei of the epithelium (*2*). In rat metaphyseal bone, 25-hydroxyvitamin D_3 was found "in epiphyseal hypertrophic cells, epiphyseal matrix, osteoid, osteoblasts, and osteocytes" (*3*) with an autoradiographic procedure that is not suitable for determining the distribution of steroids (*4*). Our dry-mount and thaw-mount autoradiographic techniques (*4*), which exclude liquid fixatives, embedding media, and dehydration fluids, have been used successfully for determining the cellular and subcellular distribution of steroid hormones (*5*) and have facilitated demonstrations of steroid hormone target cells in the brain, pituitary, thymus, heart, blood vessels, ovary, testis, and skin (*5, 6*). In the experiments described here we injected 1,25$(OH)_2D_3$ into rats and obtained autoradiographic evidence for nuclear concentration and retention of radioactivity in rat duodenum, jejunum, ileum, colon, stomach, kidney, skin, pituitary, and parathyroid.

Six 28-day-old male Sprague-Dawley rats were placed on a vitamin D_3–deficient diet for 35 days; the diet was supplemented with vitamins A, E, and K (*7*). All animals were injected intravenously with 1,25$(OH)_2$-[26,27-^{3}H]D_3 dissolved in 75 percent ethanol with isotonic saline. Two animals received 0.19 μg of the labeled compound (specific activity 90 Ci/mmole) per 100 g of body weight; and four animals received 0.16 μg/100 g (specific activity 160 Ci/mmole). The animals of the former group were killed 1 or 3 hours and those of the latter group 2 hours after the injection. Two of the rats that received the high dose were each injected subcutaneously with 1.0 μg of unlabeled 1,25$(OH)_2D_3$ 15 minutes before they were injected with the labeled compound. In addition we used one pregnant rat (*8*) that received the vitamin D_3–deficient diet from the start of pregnancy. On day 18 of pregnancy, the animal was injected intravenously with the labeled compound (0.18 μg/100 g of body weight; specific activity 160 Ci/mmole) dissolved in 70 percent ethanol (with isotonic saline) and killed 4 hours afterward. Tissues were excised, mounted, and frozen onto tissue holders, sectioned at 4 μm, and thaw-mounted on photographic emulsion (Kodak NTB3) coated slides. The autoradiograms were exposed for 3 to 11 months, then photographically processed and stained with methylene blue and basic fuchsin. The thaw-mount autoradiographic technique and the preparation of controls against chemography have been discussed (*4*). Cells are considered labeled when the

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