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Homeothermic Development of the Young Chick.* (32075)

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Newly hatched domestic chicks are poor thermoregulators but rapidly develop thermoregulation(1,2). The functional basis of this ontogenetic development of thermoregulation is not known. The increase in insulation by the growth of down and feathers, and the decrease in the ratio of surface area to body mass, both of which occur during the first week of rapid growth, generally are accepted as important contributions to effective thermoregulation by the young chick. Maturation of neural and hormonal mechanisms, and an increase in rate of thermogenesis are other suggested contributors(3,4,5). In our experiments, homeothermic development of the chick, and the effects of increases in body weight, food intake, and down on this development were studied.

Methods and procedures. Arbor Acre x Vantress chicks, with known hatch times, were used. Some chicks were hatched in our laboratory; each chick was banded and the time of hatch recorded within one hour after emergence from the shell. Other chicks were obtained from a commercial hatchery with a hatch time range of one hour. All chicks were reared in communal cages of a 35°C brooder. Three groups of chicks were used in these experiments: (a) normal chicks reared with food and water available *ad lib* in the brooder; (b) normal chicks reared for 40 hours without food and water; and (c) "shaved" chicks with the down removed from their bodies by the topical application of calcium thioglycolate 5 hours before cold stress. The shaved chicks were reared with food and water available *ad lib*.

In the first experiment, 95 free-feeding normal chicks of 5 hatch times (8-, 16-, 32-, 64-, and 128-hrs. old) were taken from the brooder at different times, weighed, and placed in a lighted incubator set at 35.5°C. One hour later, the chicks were removed quickly from the incubator and housed individually in small boxes (15 × 15 × 15 cm) in a 10°C control temperature room. Cloacal temperatures were recorded immediately from a Yellow Spring Telethermometer connected to a calibrated tissue implantation probe (Yellow Springs Model 511) that was inserted manually 2.5 cm in the cloaca. Every 15 minutes, thereafter, cloacal temperatures were measured during a one-hour exposure period. The probe readings were later converted to temperature values.

In the second experiment, 12 chicks reared without food and water until they were 40 hours old were studied. Sixty-four "shaved" free-feeding chicks of 3 different hatch times were used in the third experiment. Handling and recording procedures were the same for the 3 experiments.

Results and discussion. The cooling curves for the 5 groups of free-feeding normal chicks

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TABLE I. Means (M) and Standard Errors (SE) of Body Weights, Surface Area/Body Weight Ratios, and Cloacal Temperatures for Chicks of 5 Ages After a One-Hour Exposure to a 10°C Environment.

Age (hr)	No. of chicks	Wt (g) M	Wt (g) SE	Cloacal temp. (°C) M	Cloacal temp. (°C) SE	Relative surface area/body mass ratio* M
8	13	43.3	.8	24.1	.5	2.85
16	16	41.3	.7	27.8	.4	2.89
32	18	42.6	.8	31.8	.5	2.86
64	22	47.1	1.0	35.0	.3	2.77
128	26	57.0	1.2	37.3	.4	2.60

* Surface area calculated using the formula: surface area $(\text{meter})^2 = \text{wt } (\text{kg})^{2/3}(3)$.

are presented in Fig. 1. It is evident from these data that chicks show a rapid development of thermoregulation the first 5 days after hatching, with significant changes apparent even during the first day and one-half. For example, the 8-hour old chicks have a 14°C net drop in cloacal temperatures at the end of the one hour exposure, whereas the 32-hour chicks fall only 7.6°C during the same period. The 64- and 128-hour animals are more capable of maintaining their body temperatures during the cold stress; the cloacal temperatures of the oldest chicks fall only 0.1°C during the last 15 minutes of the exposure.

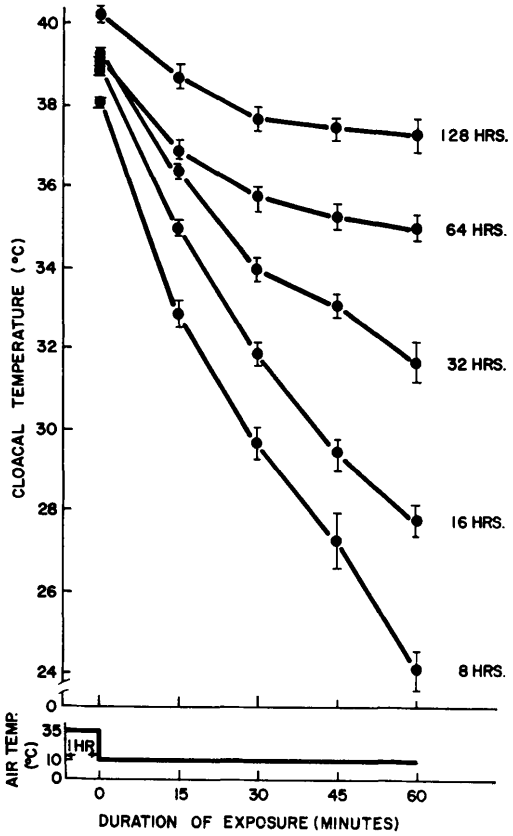

FIG. 1. Cloacal temperature changes for chicks of 5 ages exposed to 10°C. Each point represents mean ± standard error.

This development of thermoregulation cannot be explained by body weight increases. As shown in Table I, the 8-hour chicks weigh 43.3 g and have a mean cloacal temperature of 24.1°C after the one hour exposure. The 16-hour chicks, weighing 2 g less, have a mean cloacal temperature 3.7°C higher than the younger chicks. The 32-hour chicks, intermediate in body weight of the 3 youngest groups, show about half the temperature fall of the youngest animals. Significant increases in body weights are found for the two older groups, but the relative surface area to body mass ratios for all groups vary only between 2.60 and 2.89. This small ratio change cannot explain the large differences in temperature drop observed between the younger and older chicks.

The finding that 16- and 32-hour old chicks have slightly lower body weights than 8-hour old chicks is probably because of the depletion of the yolk sac without compensatory food intake. To determine if the onset of feeding or the specific dynamic action of food could account for the significant differences in cooling among the 3 youngest groups, the 12 unfed chicks were exposed to the 10°C environment when they were 40 hours old. These chicks, weighing 37.8 g, had a mean cloacal temperature of 32.6°C at the end of one hour, were 0.8° higher than the normal 32-hour old chicks, and were 7.5°C higher than the 8-hour old chicks.

Although increases in body weight and food intake cannot explain the rapid development of thermoregulation of the young chick, small changes in insulation might account for the observed differences among the age groups. An increase in the density of the down or piloerection of the down by the older chicks would provide more insulation for heat conservation. To determine the role of down on homeothermic development, 8-, 32-, and 128-hour old "shaved" chicks were studied.

The "shaved" chicks (Fig. 2) cool significantly faster than normal animals of the same age (Fig. 1). However, the relative ability to regulate body temperature for the shaved chicks of different ages was the same as the normal chicks. Thus, among the shaved chicks, the 8-hour old group cools fastest; the 32-hour old chicks, weighing less than the 8-hour old chicks, were about 3°C higher at the end of one hour; and the 128-hour old chicks were 7.4°C higher than the 32-hour old chicks. As a body weight control, five 128-hour old shaved animals were sacrificed immediately after the first reading. The cooling of these dead chicks was significantly greater than any of the live, shaved groups (Fig. 2). These results show that the down contributes significantly to the chick's thermoregulation as indicated by the cooling differences between the shaved and normal chicks of the same age. However, the rapid development of homeothermy in the young chick cannot be explained by insulation increases since significant differences occur among shaved animals of different ages.

In some mammals (rats, cats, dogs) the ability to maintain body temperatures during cold stress develops slowly the first 2 to 3 weeks after birth(6,7,8). The rat, for example, must be 35 to 40 days old before its normal body temperature can be maintained for one hour in a 10°C ambient temperature (6). The precocial guinea pig, however, is capable of maintaining its body temperature immediately after birth(9). In most mammalian species the newborn, when exposed to cold, shows a minimal amount of shivering. As the animal matures, shivering increases and non-shivering thermogenesis decreases. This early non-shivering thermogenesis seems

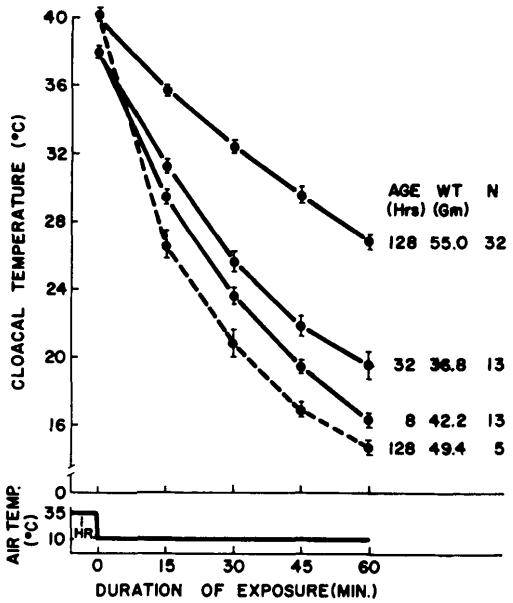

FIG. 2. Cloacal temperature changes for shaved chicks of different ages exposed to 10°C. Each point represents mean ± standard error. The dashed line represents 128-hour-old shaved chicks sacrificed immediately after the first reading.

to be related to sympathetic neural activity and the presence of brown fat(9,10). Experiments on metabolic changes during the chick's rapid development of homeothermy are in progress.

Summary. One-to 5-day old chicks were exposed to a 10°C ambient temperature and cloacal temperatures were recorded every 15 minutes for one hour. Young chicks show a rapid development of thermoregulation the first 5 days after hatching, with significant changes apparent even during the first day and one-half. This rapid homeothermic development is correlated with age and cannot be explained by increases in body weight, food intake, or insulation.

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The Antiviral Activity of Caprochlorone. (32076)

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Although many different compounds have been shown to suppress viral replication *in vitro,* caprochlorone [levo-4-phenyl-4-(2-chlorobenzyl)-5-oxy-hexanoic acid] is one of the few which provides consistent activity against a viral infection in animals(1,2). A prophylactic treatment regimen produced an increase in survival of mice infected with a strain of influenza A/PR8. Mean survival time of mice was increased, and virus content of lungs was suppressed. Inhibition of virus HA titers was also demonstrated in embryonated and deembryonated eggs infected with the same strain of influenza; activity was ascribed to intracellular inhibition both early and late in the infectious process(2,3).

Paucity of information on the spectrum of activity of caprochlorone and its action mechanism prompted the present investigation using cell culture systems. Studies with antiviral compounds l-adamantanamine·HCl (4,5,6) and ammonium phosphate(7,8,9) were carried out in conjuction with those using caprochlorone to better elucidate the site of compound action.

Materials and methods. Viruses. Influenza strains A/WSN, A/PR8, and B/Maryland were used for action mechanism studies. The former was obtained from W. Henle, the latter 2 from the American Type Culture Collection. Viruses were propagated in embryonated eggs; virus in allantoic fluid was partially purified by 2 cycles of centrifugation with sedimentation at 26, 700 $\times$ *g* for 1 hour and resuspension in phosphate buffered saline (pH 7.6; PBS) containing 0.1% bovine serum albumin (PBS/BSA). Other viruses, used for antiviral spectrum studies, were grown in appropriate cell culture systems or embryonated eggs. All specimens were stored at —60°C prior to use.

Compounds. Caprochlorone, l-adamantanamine·HCl and dibasic ammonium phosphate were employed. Solutions were prepared in maintenance media; pH of media was adjusted to 7.6 by appropriate addition of NaOH or HCl.

Virus antisera. Antisera to influenza strains A/WSN, A/PR8, and B/Maryland were prepared in adult White Leghorn roosters. Ten ml of infected allantoic fluid was inoculated intravenously, and 2 ml intramuscularly, into each bird. After 7 days, birds were similarly reinjected. Ten days later serum was collected. Prior to use, serum was mixed with freshly trypsinized chick embryo (CE) cells (2×10^7 cells/ml of serum) for 2 hours at 37°C to adsorb non-viral antibody. The serum, clarified by centrifugation, was heated to 56°C for 30 minutes and then treated with potassium periodate(10) to remove non-specific inhibitors. Hemagglutination inhibition tests, using 4 units of corresponding virus, provided titers of 1024, 512, and 640 for A/WSN, A/PR8, and B/Maryland, respectively.

Media. Liquid medium for maintenance of CE cell cultures (MM) was composed of Earle's saline with 0.5% lactalbumin hydrolysate, 0.1% yeast extract, 10% tryptose phosphate broth, 2mM L-glutamine, 100 units/ml penicillin G, and 100 μg/ml streptomycin·SO_4. Medium for plaque inhibition tests with human embryonic lung (HEL),