

ZOOLOGY

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ABSTRACTS

The Krushinsky Apparatus: A Test for Self Awareness in Farm Animals. J. L. ALBRIGHT, Department of Animal Sciences, Purdue University, West Lafayette, Indiana 47907 and R. Kilgour and W. G. Whittlestone, Ruakura Animal Research Station, Private Bag, Hamilton, NZ.—The view that man is unique in the possession of language and that animals other than man have no mental experiences has been challenged by D. R. Griffin (*The Question of Animal Awareness: Evolutionary Continuity of Mental Experiences*. The Rockefeller University Press, New York, NY, 1976). Arguments persist and those interested in the welfare of farm animals are concerned by what animals would choose if presented the opportunity. One such early test to determine how animals think, anticipate, and react was developed by Krushinsky and his Russian colleagues (Krushinsky, L. V. 1965. *Solution of Elementary Logical Problems by Animals on the Basis of Extrapolation*. *Progress in Brain Research* Vol. 17. Cybernetics of the Nervous System. Edited by Norbert Weiner and J. P. Schade. Elsevier Publishing Co., New York, NY. p. 280-308). With pigeons, hens, crows, rabbits, cats and dogs, they utilized the principle of food placed in one of two feeding bowls which stood side by side at the gap behind a screen. When an animal starts to eat the food, both bowls were moved in straight lines in opposite directions. After covering a distance of 20 cm, they disappeared behind non-transparent flaps. To solve the problem, the animal had to run around the screen on the side behind where the feeding bowl had disappeared. Their animals had success rates of: pigeons (7%), hens (52%), crows (86%), rabbits (27%), cats (86%) and dogs (89%). We adapted Krushinsky's technique to farm animals so as to provide a slow-moving trolley carrying food within a large animal learning maze. It disappeared into an A frame tunnel and, after certain periods of time, reappeared at the other end.

Three dairy cows were tested. When the food trolley moved, they demonstrated a "startle" reaction (staring at the object with front feet firmly planted) which may have inhibited the learning process. It took them two weeks before they learned to continue eating from the moving food box and anticipate the reappearance of the trolley. After our presence was removed, success came about by observing the cows with a TV monitor. The cows had become conditioned and dependent upon us expecting us to remove them from the testing arena after the food disappeared. They explored and solved the problem *only* after we were out of sight. Since cows learn from each other, each cow and later each bull were put in the testing arena separately. Earlier, another 10 to 11 cows "startled" when the food object started moving and they did not learn how to solve the problem.

None of eight dairy bulls (four Jersey and four Friesian) learned to anticipate and had a larger startle response than cows. They stopped eating once the food box was set in motion. They showed a startle reaction when the trolley was 9 to 10 m

away from them. A study of spatial distances between animals and between a particular animal to a small mechanical moving object in the field was undertaken with the same dairy bulls. Bulls will stand quietly without fighting if greater than 6 m apart; then at about 5-6 m the bulls would back away (as this inanimate object had violated their body space) with escape at a distance of 3 m.

In addition, 12 out of 15 pigs were able to "extrapolate" from a known situation within 3 days, and six out of seven rams tested in 4 days.

Localization of Glycoproteins in Prepubertal Mouse Ovaries. LARRY R. GANION, Department of Physiology and Health Science, Ball State University, Muncie, Indiana 47306.—Glycoproteins serve a variety of functions in biological tissues. To localize glycoproteins associated with ovarian follicles, ovaries from prepubertal, HS, mice were rinsed in Tyrode's solution, bisected, cultured at 37°C *in vitro* in BGJB medium containing H^3 glucosamine (500 μ Ci/ml) for 13-180 minutes. Next, the tissues were rinsed and fixed for 2 hours in 3% glutaraldehyde in 0.1M phosphate buffer (pH 7.2). After postfixation in 1% osmium tetroxide buffered with 0.1M phosphate buffer (pH 7.2) for 1 hour, the tissues were dehydrated in a graded series of alcohols, treated with propylene oxide and embedded in Epon 812. Thick epon sections were prepared, coated with Kodak NTB-2 emulsion and exposed for 4-6 weeks. Ultrathin sections were cut, transferred to celloidin-coated slides, stained with 2.5% aqueous uranyl acetate, carbon coated, coated with Ilford L-4 emulsion and stored for several months. After development in Dektol and Microdol-X respectively, the thick sections were stained with basic fuchsin and the ultrathin sections were transferred to grids for viewing with an RCA electron microscope. As revealed by examination of the light microscope autoradiographs, the most active site of H^3 glucosamine incorporation is the theca, which becomes black with label after 3 hours of culture. With the electron microscope, silver grains are seen associated with the endoplasmic reticulum and Golgi bodies of thecal cells. The collagen fibers situated between thecal cells are also labeled. In the smaller unilaminar ovarian follicles, most of the label is associated with the follicular envelope. In some of these follicles, as evidenced by electron microscopy, the zona pellucida is noticeably tagged with H^3 glucosamine. Both the oocyte and follicle cells of multilaminar ovarian follicles readily incorporate H^3 glucosamine. Frequently, the zona of such follicles is extensively labeled. Of interest is the association of label with the chromatin of thecal and follicular cell nuclei. These data indicate that glycoproteins are major constituents of the mammalian ovarian follicle. Further, it is speculated that the collagen of the theca is elaborated by the theca cells and the zona is a product of both the follicular envelope and oocyte.

Effects of Triamcinolone Acetonide on the Metabolism of Phenolic Compounds in the Adult Female Mouse. R. R. HALL and K. L. ESBENSHADE, Indiana University—Purdue University at Fort Wayne, Fort Wayne, Indiana 46805.—In the first of three experiments, sixty mature, female, ICR mice were randomly divided into a treated ($n=30$) or control group ($n=30$). The mice in the treated group received 80 μ g triamcinolone acetonide (TA) at -4 and 0 hr. Each group was further divided into 5 subgroups consisting of 6 mice each and given 0.12, 0.15, 0.19, 0.24 or .29 mg p-cresol suspended in oil per g body weight at 0 hr. All substances were given IP. The LD_{50} for control and TA-treated animals was 0.19 and 0.15 mg/g, respectively. The rate of excretion of phenolphthalein (P) after an IP administration was examined in experiment 2. Six female mice served as controls and six others were given 100 μ g TA at -12 and 0 hr. All animals received 5 μ moles P at 0 hr, placed into metabolism cages and feces and urine collected at 4, 10 and 24 hr. Urine

and homogenized feces were divided into two aliquots. One aliquot was incubated with 500 Fishman Units B-glucuronidase for 60 min. at 37°C. P was determined spectrophotometrically on both aliquots following extraction with ether. The accumulative amount of unconjugated P and P-glucuronide excreted in the urine at times 4, 10 and 24 hr for control was 280, 530 and 1170 and 2100, 3080 and 3930 and for TA-treated was 170, 550 and 1040 and 1220, 1410 and 2850 nmoles, respectively. In experiment 3, 8 mature, female mice were randomly divided into a control (n = 4) or a treated group (n = 4) to observe the effect of TA on uridine diphosphoglucuronyl transferase (UDP-GT) activity. The animals in the treated group were given 80 ug TA twice daily for 2 days. A hepatic homogenate was prepared from each animal and incubated at 37°C with 75mM maleate buffer (pH 6.4), 0.05% triton X-100, 0.5mM p-nitrophenol and 1mM UDP-glucuronic acid. Aliquots were taken at 20 min intervals and analyzed for p-nitrophenol. The UDP-GT activity in control and TA-treated animals was 115 ± 14 and 59 ± 13 nmole/hr/mg tissue ($\bar{X} \pm SE$), respectively. The results indicate that TA, a synthetic glucocorticoid, retards the glucuronide conjugation of phenolic compounds.

Fine Structure of the Pig Harderian Gland. MOHINDER S. JARIAL, Department of Physiology and Health Science and Muncie Center for Medical Education, Ball State University, Muncie, Indiana 47306.—The Harderian gland of the adult pig is distinctly separated from the more superficial nictitans gland of the third eyelid and is located inferolaterally deep in the orbit in close proximity to the periorbita.

Histologically the Harderian gland of the pig is a tubuloacinar gland composed of serous acini and ducts. Based on the density of the cytoplasmic matrix, two cell types can be distinguished in the acini and intralobular ducts, i.e., "dark" and "light" cells. The dark cells in contrast to light cells contain extensive granular endoplasmic reticulum and dilated smooth endoplasmic reticulum, numerous Golgi complexes, PAS positive granules of different densities and vacuoles in the cytoplasm. Both the dark and light cells contain numerous mitochondria and dense bodies and their luminal surfaces have few, short microvilli. The lateral plasma membranes of both cell types are joined by occluding junctions and often contain intercellular channels lined with microvilli. The myoepithelial cells are often present in relation to both cell types. The intralobular ducts are lined with dark and light cells, which appear structurally similar to those of the acini, but contain relatively fewer secretory granules. Since the dark and light cells of the acini and ducts differ only in the amount of endoplasmic reticulum, Golgi complexes and the number of secretory granules, it is suggested that they represent different functional stages of a single secretory cell type.

In the acinar portion of the gland numerous granules, dense bodies and vesicles mainly from the dark cells are secreted in whole and retain their membranes after extrusion into the acinar lumina. Since cytoplasmic fragments have been observed in the ductal lumina, it appears that the secretion there is liberated by the apical extrusion and fragmentation of the ductal cells.

In conclusion, the Harderian gland of the pig is a typical serous gland which has an "apocrine" mode of secretion.

Mebendazole Therapy of Enteral Trichinellosis in Mice. R. O. MCCracken, A. GARCIA, and H. G. ROBINS, Department of Biology, Indiana University-Purdue University at Indianapolis, Indianapolis, Indiana 46205.—Recent reports have demonstrated the chemotherapeutic effectiveness of a benzimidazole anthelmintic, mebendazole, in the treatment of *Trichinella spiralis* infections in rats and mice.

Because mebendazole (methyl 5-benzoyl-2-benzimidazolecarbamate) is used for the treatment of other helminthoses in man, and because it is highly active against enteral and parenteral forms of *Trichinella* in laboratory animals, this compound is one of great potential utility in the treatment of human trichinellosis. However, there have been comparatively few studies of the susceptibility of *T. spiralis* to mebendazole treatment during the enteral phase of infection or of the efficacy of dosage regimens similar to those used in man. Data published by Fernando and Denham (1976, J. Parasitol. 62: 874-876) and McCracken (1978, J. Parasitol. 64: 214-219) suggested the possibility that, as with thiabendazole, parbendazole, and cambendazole, the *Trichinella* population becomes progressively less susceptible to anthelmintic treatment as the worms mature in the gut. For example, a single oral dose of mebendazole at 50 mg/kg 7 hr after inoculation eliminated approximately 95% of the immature worms; the same dosage was only moderately, but significantly active at 72 hr after inoculation when the larvae would have become adults.

We sought here a treatment regimen with mebendazole that would be effective against the drug-resistant adult worms. Mebendazole proved to be highly effective against adult *T. spiralis* in a 3-day treatment course during the intestinal phase of experimental trichinellosis in mice. When treatment began 72 hr after the mice were infected with parasites, the number of adult worms recovered from the host intestine was greatly reduced (93%) by twice daily oral doses of mebendazole at 7.5 mg/kg body weight. (Supported in part by a summer faculty fellowship from the Purdue Research Foundation to R. O. McCracken)

Effects of Prolonged Starvation of Male *Ambystoma tigrinum* in Post-Reproductive Condition. ERIC D. MOULD and DAVID M. SEVER, Department of Biology, Saint Mary's College, Notre Dame, Indiana 46556.—Anorexia and involuntary starvation represent a significant component of the life history of *A. tigrinum*. We investigated the effects of extended starvation upon male *A. tigrinum* by maintaining them in aquaria without food for a period of up to 70 days. Lipase activity increased significantly during each of the first four weeks of starvation. At 35 days lipase activity reached a maximum level and did not increase in the animals starved for 70 days. Hepatic glycogen steadily decreased from an average value of 3.32g glycogen/100g liver at the time of capture to less than 0.50g glycogen/100g liver after 70 days. Visible fat body content varied erratically for the samples of this study and showed no clear pattern except for the very significant reduction in mean fat body weight from the time of capture to the 70 day measurements. Body weight decreased to 45.5% of the initial weight during the study but, as was the case for fat body, did not show a sequential decrease among samples. Gonadal tissue was catabolised from 1.64% of body weight to 0.25% of body weight within 21 days of starvation and shows no further reduction as a percentage of body composition. Although absolute liver weight decreased during the study, it remained an extremely consistent $2.66 \pm 0.22\%$ of body weight throughout the entire study.

A Preliminary Study of the Effects of Sidestream Smoke on Postnatal Development and Growth in Mice. JAMES M. REYNOLDS and CHARLES E. MAYS, Department of Zoology, DePauw University, Greencastle, Indiana 46135.—Female albino (COX-SWISS) mice were exposed to sidestream smoke, and the effects on postnatal development and growth of their offspring were studied. Experimental female mice were placed in a 3.0 cm x 20.5 cm x 25.0 cm perforated plexiglass box and ex-

posed to the smoke of one filter cigarette (Marlboro, softpack) until it had burned to the filter. This method of delivering tobacco inhalants, referred to as complete chamber exposure, took approximately 15 minutes. An additional 30 minutes was allowed for the smoke to clear from the box. After the 45 minute period, the mice were returned to their cages. Smoking of the experimental females began on the same day that they were paired with a male, and was continued once each day until they gave birth to their litters. Control female mice were placed in a similar box for 45 minutes but were not exposed to a burning cigarette. After parturition, the mothers were allowed to nurse their young, undisturbed, until weaning them at day 21. Approximately one month after weaning, both experimental and control mothers were followed through a second litter under the same conditions as those used for the first litter.

Control mice had an average of 11.8 progeny per litter, whereas the average litter of the experimental mice was 8.6 progeny. Percent survivorship at day 21 (weaning) was determined. An average of 9.6 control mice out of the mean litter size of 11.8 survived to weaning, resulting in an 81 percent survivorship. Experimental mice had only a 72 percent survivorship with an average 6.2 progeny out of a mean litter size of 8.6 surviving to weaning. The male to female sex ratios at weaning were 1:00 (46.0%): 1.17 (54.0%) for the control mice and 1:00 (48.8%): 1.05 (51.2%) for the experimental mice. No significant differences were noted in the growth data. However, the control mice were slightly larger than the experimental mice at birth and the experimental mice were slightly larger at weaning. The latter may be caused in part because there is an average of 3.4 more control mice than experimental mice at weaning. This may create a greater amount of progeny competition for milk. In addition, there was a greater percentage of experimental males than control males that survived to weaning, and male mice are generally larger than female mice. These data indicate that sidestream smoke causes reduced litter sizes and lower percent survivorship in COX-SWISS mice.

The Subspecies of the North American Brown Creeper. J. DAN WEBSTER, Department of Biology, Hanover College, Hanover, Indiana 47243.—Thirteen races are recognizable; they are differentiated by color, wing length, and bill length. Geographical variation is clinal, with abrupt steps at a few lines. Migration is fairly regular over long distances in the eastern race, but altitudinal or irregular as to distance and direction in the western and southern Appalachian races. The Mexican and Central America races are resident. A suggestion by a student of bird songs that there may be sibling species in North America led to a search for possible differentiating morphological characters; none were found.

Investigation of Maternal Pheromones in *Octodon degus*. PAMELA J. WHITE, ROBERT B. FISCHER, and GARY F. MEUNIER, Ball State University, Muncie, Indiana 47306.—*Octodon degus* are diurnal hystricomorph rodents, precocial at birth and inhabit a complex system of burrows with a social organization based on group territories. The young are apparently reared by several females in a common nest. The focus of this experiment was to investigate the possibility that infant *degus* respond to maternal olfactory cues. The first study examined whether *degus* pups recognize a lactating female. The infants did not show a preference for bedding from a novel lactating female when allowed to choose between it and bedding from either their lactating mother, a novel non-lactating female, a male, or unsoiled bedding. The second experiment investigated whether they would discriminate between their lactating mother and a novel lactating female when each was present in the situation. Given this choice, the *degus* pups spent significantly more time in

proximity to the novel female. The basis for this choice is unknown but may be related to their extreme precocity. Also, because the young are reared in communal nests, they may not be under any selection pressures to discriminate between mothers. This idea is aided by the fact that the degu shares its burrow system with a separate genus *Abrocoma bennetti*.