ZOOLOGY

Chairman: Thomas Joseph, Department of Biology
Indiana University-South Bend, South Bend, Indiana 46615

Chairman-Elect: John O. Whitaker, Jr., Department of Life Sciences
Indiana State University, Terre Haute, Indiana 47807

ABSTRACTS

Numerical Taxonomic Studies of 336 Human Diseases, Using 82 Symptoms. James Fernandez, Joseph Mather, and Theodore Crovello, Biology Department, University of Notre Dame, Notre Dame, Indiana 46556.——Numerical Taxonomy has been described as the numerical evaluation of the affinity or similarity between taxonomic units and the ordering of these units into taxa on the basis of their affinities. We employed procedures of numerical taxonomy to study the relationships among human disease based on 82 symptoms. In addition, we analyzed the relationships among the symptoms as well, to try to delimit meaningful clusters of symptoms. Our data base, a matrix of 336 diseases and the presence or absence of 82 sysmptoms, was obtained from LOGODX, a computer-assisted disease diagnosis system, available commercially from Pelam Inc., Chicago, Illinois 60625. A simple matching coefficient was calculated for all possible pairs between the 336 diseases. These results formed the basis for a cluster analysis using the Unweighted Pair-Group Method. The resulting tree, with its disease groupings, was then compared with conventional ideas of disease classification. As a separate study, we also took a small group of childhood diseases and compared the clustering results of using 82 versus 15 symptoms. To determine the effects of negative matching, we used Jaccard's coefficient of association, and compared the results with those previously obtained with the simple matching coefficient. Our studies have produced some interesting conclusions about the grouping of disease and the inter-relationships between symptoms.

Locomotor Activity Response of *Peromyscus leucopus* to Accelerated Days. Bryan Flueckiger and Don R. Taves, Indiana University-Purdue University at Fort Wayne, Fort Wayne, Indiana 46805.——Locomotor activity patterns of six male *Peromyscus leucopus* exposed to artificial 21-hour light:dark days indicated that the animals were unable to entrain to this light strategy. Five of six animals underwent periodic phase shifts which resulted in their confining activity to the dark portion of each period. Four control animals successfully entrained to 24-hour light periods.

The Effects of Chlorine on the Development of *Rana pipiens* Eggs: Preliminary Results. Betty I. Tarnowski, Butler University, Indianapolis, Indiana 46208.——Using a continuous flow system, *Rana pipiens* eggs were exposed to chlorine concentrations of 0.04 ppm, 0.3 ppm, 0.6 ppm and 0.9 ppm every 12 hours, for 1 hour duration, for a total of 9 days

(218 hours). Chlorine concentrations were measured by amperometric titration. Prior to hatching, stage 19 (Shumway, 1940), no differences were noticed between the test and the control organisms. Effects of chlorine exposures were observed to have occurred during stages 19-25: embryos exposed to 0.9 ppm and 0.6 ppm chlorine showed a 100% kill following hatching while exposure to chlorine concentrations of 0.3 ppm and 0.04 ppm resulted in a slower developmental rate in addition to reduced survival.

Cytology of an Agametic Gonad Condition in *Drosophila melanogaster.* STEPHEN W. STONER and LEE ENGSTROM, Biology Department, Ball State University, Muncie, Indiana 47306.——One of the earliest visible differentiative events to occur during the development of *Drosophila* is the precocious separation of pale cells from the posterior end of the egg. Some of these pole cells later become the germinal cells of the adult reproductive organs. A strain originally collected in Marguarita Island, Venezuela which possessed temperature-sensitive genetic influences resulting in 20% of the individuals having unilateral or bilateral agametic gonads. Cytological evidence will be presented indicating that adult ovaries and testes possess normal mesodermal components and are attached normally to the germinal disc components; however, no germinal elements are present. We conclude: (1) the genetic influences resulting in agametic gonads prevent normal pole cell differentiation and (2) the formation of all other components of the reproductive systems are under genetic controls distinct from those controlling the germinal elements.

Influence of Gonadal Hormones on RNA Populations Found in the Adrenal Gland of White Mice. WILLIAM P. SHOFNER and TIMOTHY A. STABLER, Department of Biology, Indiana University Northwest, Gary, Indiana 46408.——Stabler and Ungar (Endocrinology, 86:1049, 1970) demonstrated that estradiol, when administered to male white mice, would induce activity of the adrenal enzyme, 20 α-hydroxysteroid dehydrogenase, Testosterone, when administered to nulliparous female white mice, would block such activity. Histochemical studies located the enzyme to be found in the X-zone of the adrenal. Stabler and Wotiz (Am. Zool., 12:675, 1972) demonstrated that the mouse adrenal gland has a specific estrogen receptor. However, this receptor does not follow the accepted model of steroid hormone action as it does not enter the nucleus to interact with the genome.

In an attempt to determine if the receptor-hormone complex is working in the cell cytoplasm at the level of RNA, groups of hormone treated and control animals were set up for both sexes. The adrenals were removed, the RNA extracted using cold phenol and the extracts subjected to polyacrylamide gel electrophoresis at 4° C. The gels were then stained with methylene blue and photographed for record.

Comparison of the gels indicated no differences between groups and/or sexes. It is thus concluded that RNA output is similar in each case. Work is now progressing on enzyme forms that may be present in the adrenal cells.

Stabilization of Lung Compliance in Rabbits During Natural and Artificial Respiration. THOMAS A. LESH and ANTHONY R. DOWELL, Department of Physiology and Health Science and Center for Medical Education, Ball State University, Muncie, Indiana 47306.——The "sigh" reflex in mammals periodically provides a brief hyperinflation which reopens collapsed alveoli and prevents undue decreases in lung compliance (Bartlett, D., 1971. *Respiration Physiology* 12:230-238). We made a quantitative study of this effect in New Zealand White rabbits, pretreated with acepromazine and anesthetized with sodium pentobarbital. In 15 measurements on 5 spontaneously breathing animals, a single "sigh" increased lung compliance by 6.6 $\pm$ 5.8 (SD) per cent; this change was significant ($P < .001$ by Student's t-test). The interval between sighs was 11.3 $\pm$ 2.5 (SD) minutes. Sighing could be suppressed by deepening anesthesia and applying intermittent positive-pressure ventilation (IPPV) at a rate sufficient to override spontaneous respiratory efforts. In 10 trials, compliance had fallen by 20.3 $\pm$ 12.9 (SD) per cent after one-half hour of IPPV ($P < .005$). An artificially induced mild hyperinflation of the lungs for one respiratory cycle raised compliance markedly but not always fully to the control value. Compliance rose further upon repeated hyperinflations or simply a return to spontaneous respiration.

Blood Pressure Studies Following Unilateral Ureteral Ligation in Rats. W. J. EVERSOLE, Indiana State University, Terre Haute, Indiana 47809. ——The results of this study demonstrate that severance or ligation of the right ureter leads to increases in blood pressure in rats drinking 1% saline. The pressure is elevated at three weeks following operation and rises thereafter reaching a mean in some groups in excess of 200mm Hg by seven weeks. Ligation of the ureter approximately 3 centimeters from the hilum results in a greater rise in pressure than ligation near the hilum. Severance of the right ureter distant from the hilum also leads to extremely high pressures. Proximal to the ligation or severance point there is accumulation of fluid and eventual complete degeneration of the right kidney. Injection of fluid obtained from the degenerate renal pocket usually causes a rise in blood pressure in intact test animals, leading us to postulate the presence of a hypertensive agent in the accumulated fluid. Hypertensive animals have enlarged hearts and kidneys and in some cases the left kidney is grossly diseased.

Effects of an Unknown Factor on the Development of Adrenal Regeneration Hypertension. C. L. DEMAIO and W. J. EVERSOLE, Indiana State University, Terre Haute, Indiana 47809.——Rats derived from the Charles River strain were subjected to operative procedures which usually lead to the development of cardiovascular hypertensive disease within 5 weeks. One hundred seven rats were used in this study and were treated as follows: (I) right adrenalectomy; (II) right adrenalectomy, left adrenal enucleation; (III) right adrenalectomy, left adrenal enucleation, and right partial nephrectomy; (IV) right adrenalectomy, left adrenal enucleation and right nephrectomy; (V) right adrenalectomy, left adrenal enucleation, right nephrectomy and left partial nephrectomy. Following the operation most animals exhibited poor appetite, lassitude, muscular weakness and reduced weight gain. The mortality was excessive

and correlated with the severity of the operation. The per cent surviving seven weeks in each group was 83, 83, 62, 29, and 11 respectively. In prior experiments the survival, regardless of type of operation, was over 98%. Animals in group IV were expected to gain weight at a near normal rate and at least 80% of them should have been hypertensive (BP>149mmHg), exhibiting enlarged kidneys and hearts at seven weeks post operative. However, 70% of the animals in this group died and 80% of the survivors had normal blood pressures but their hearts were enlarged. Four survivors in Group V exhibited a mean pressure of 169 mmHg and their hearts and kidneys were larger than those in other groups. It therefore appears that these animals suffered from a nutritional deficiency although the possibility of a latent infection has not been ruled out.

Effect of Aminoglutethimide on Rabbit EKG and Blood Pressures. MERVIN C. YODER and WILLIAM J. BRETT, Indiana State University, Terre Haute, Indiana 47809.——This study was conducted to determine if aminoglutethimide (AG) is acting on the sympathetic and/or vagal regulation of the rabbit heart to produce changes in blood pressure and heart rate. The right femoral and right common carotid arteries were exposed and cannulated in sodium pentobarbital anesthetized female rabbits to obtain direct systemic and intraventricular blood pressure. Heart rate was continuously monitored along with these pressures.

Intravenous (IV) sodium pentobarbital administration resulted in significant increases in heart rate. Lighter rabbits required higher doses of pentobarbital to reach level 3 anesthesia and consequently had significantly greater increases in heart rate than heavier rabbits. A 50 mg/kg IV injection of AG produced significant parallel reductions in mean systemic and mean intraventricular blood pressures which were dependent on the rate of AG injection. Heart rate decreases were variable but significant when compared to the results of control injection of distilled water. Blockage of beta adrenergic, alpha adrenergic, and cholinergic receptors with propranolol, phenoxybenzamine, and atropine methyl bromide, respectively, did not significantly change the effect of AG on blood pressure and heart rate even though atropine and propranolol significantly decreased heart rate and phenoxybenzamine significantly decreased blood pressure prior to AG injection.

There was no significant difference in the relationship between percent decrease in mean systemic blood pressure and rate of AG injection between blocked and non-blocked rabbits. Heart rate changes were variable and significantly decreased. These results suggest that AG does not act on the sympathetic and/or vagal regulation of the rabbit heart to produce decreases in blood pressure and heart rate.

Evidence for Acetylation of Aminoglutethimide by the Rat Liver. VICKY M. WELLS and WILLIAM J. BRETT, Indiana State University, Terre Haute, Indiana 47809.——The liver and kidney of certain vertebrates have been demonstrated to function in the excretion of Aminoglutethimide (AG). This study was conducted to determine whether AG is acetylated prior to excretion.

AG and "related" compounds from rat feces and urine were extracted in methylene chloride. The extracts were run against AG and acetylated AG standards by use of thin layer chromatography and spot location was procured by viewing under visible and U.V. light. Rf values for compounds from both urine and feces suggest the presence of AG and acetylated AG. Rat liver homogenate incubated with AG and sodium acetate also demonstrated similar thin layer spots.

Mestranol Receptor-Sites as a Relationship to Altered Cell Free Proteins. GREGORY E. CAPLINGER, PETE RIDLON, and LARRY GANION, Department of Physiology and Health Science, Ball State University, Muncie, Indiana.——It was reported by Caplinger *et al.* that the use of mestranol in varying quantities caused a subsequent alteration in the ovarian synthesis of cell free proteins. Where normal dose concentrations were employed there appeared to be a deletion of amino acid residues from the cell free proteins synthesized. This was thought to be an inhibition taking place in the ovary due possibly to an over saturation of the receptor-sites involved. If this were the case then the receptor-site assay would be lower in the fraction bound as compared to the lower concentration of mestranol. This was the case in the ovary. The relative numbers of mestranol receptor-sites was lower than the other concentration of mestranol used. The receptor assays of the groups where lower quantities of mestranol were employed was much higher which shows evidence for the proposed catalysis and addition of amino acid residues to the cell free proteins assayed. Thus one can conclusively see that mestranol and in all probability many other estrogens and other hormonal compounds have the chemical ability to effect receptor-sites which will then have a subsequent affect on protein synthesis and the entire metabolic pattern.

Localization of Mestranol Receptor-Sites in the Ovary. GREGORY E. CAPLINGER and LARRY GANION, Department of Physiology and Health Science, Ball State University, Muncie, Indiana 47306.——Within the past two years there has been much interest in the localization of hormonal receptor-sites and the subsequent role they play in regulating the metabolism of the organ involved. It was recently reported that many of the estrogenic compounds main receptor-sites were found in the uterus as would be expected since the so called target organ of estrogen is the uterus. It was also found that there are two specific receptor-sites within a particular cell. There is a cytosol receptor of which most of the hormone has been found located within the nuclear bound fraction. However this work by O'Malley (1971) and Gorski (1971) dealt with a physiological system in a normal state of equilibrium. Our concern was basically with a primed system as one would normally find in the case of an administered estrogen therapy program. In this state our results were conclusive and in opposition to previously reported data. There were distinct receptor-sites located in all organs assayed which included the uterus, ovary, pituitary, and liver. The majority of the bound mestranol was localized in the uterus with a relatively high bound fraction occurring within the ovary. Of the ovarian bound fraction most occurred in the nuclear fraction. This nuclear bound fraction would

account for the abnormal alteration of ovarian cell free proteins by Caplinger *et al.* (1974). In conclusion these results of mestranol receptor-sites in the ovary support the previously published data by Caplinger *et al.* that point to altered ovarian cell free proteins as a result of mestranol treatment. Thus the interaction of mestranol, receptor-site, and subsequent metabolism are dependent upon the preexisting use of mestranol and the concentration used. Also as one can see this alteration will inevitably affect ovarian metabolism and synthesis of proteins.

An Electron Microscope and Autoradiographic Study on the Formation of the Zona Pellucida in the Prepubertal Mouse Ovary. LARRY R. GANION, Department of Physiology and Health Science, Ball State University, Muncie, Indiana 47306.——Ovaries from 1- to 21-day-old mice were fixed in 3% glutaraldehyde, post-fixed in 1% osmium tetroxide, embedded in epon 812, and viewed in an RCA EMU 3-C electron microscope. Zona pellucida development began in unilaminar ovarian follicles. Small clefts were initially observed between the oocyte and follicular envelope. These clefts subsequently became filled with a granular-fibrillar material and fused to form a continuous layer around the egg. During this time, elements of rough endoplasmic reticulum were observed within the ovarian follicle complex and were especially numerous within follicle cells. Profiles of vesicular and saccular Golgi were commonly encountered in the developing follicles. Moreover, many large vesicles were observed in the corticle ooplasm, some appearing to fuse with the oolemma. Preliminary histochemical studies suggested that the mouse zona pellucida is composed of a carbohydrate-protein complex. To investigate synthetic activities at the time of zona pellucida formation, prepubertal mouse ovaries were cultured *in vivo* with either glucose–^{3}H or leucine–^{3}H and prepared for light microscope autoradiography. Both isotopes were readily incorporated by the developing follicles. In each instance, after one day the label was generally distributed over the ovarian tissue and numerous silver grains appeared over the oocytes. With time the oocytes became extensively labeled and in some follicles, grains occurred over the zona pellucida. These observations will be discussed with respect to the origin of the zona pellucida.

A Scanning Electron Microscope Study of Female *Androlaelaps fahrenholzi* (Acarina:Laelapidae) from the Woodchuck, *Marmota monax*. REBECCA J. GOFF and JOHN O. WHITAKER, JR., Department of Life Sciences, Indiana State University, Terre Haute, Indiana 47809.——*Androlaelaps fahrenholzi* has been reported from 118 mammal species over a geographic range of 54 states and Canadian provinces in North America (Whitaker, J. O., Jr. and N. Wilson, Amer. Midl. Natur. 91:1; 1974). Its morphological variability between hosts has led several authors to consider *A. fahrenholzi* a complex of closely related species. Due to its increased surface resolution, a scanning electron microscope (SEM) study was undertaken in an effort to identify fine structural features not easily resolvable under the light microscope that might prove valuable in classification. Examination with the SEM revealed that: (1) anal aciculae of uniform length are oriented anteriorly and arranged in rows at the base of the anal plate; (2) the

stigmal pore contains randomly grouped projections and the peritreme is lined with fine peg-like structures; (3) the tritosternal pili and deutosternal denticles do engage; and (4) rows of tiny projections extend into the pre-oral groove in close association with the sensillae of the labrum. It is hoped that similar comparative studies of *A. fahrenholzi* from different hosts will aid in determining whether these forms should properly be considered as *A. fahrenholzi*, or whether some should be recognized as separate species.

The Social Behavior of a Group of Holstein-Friesian Milking Cows. JAMES C. WILSON, Hawkesbury Agricultural College, Richmond N.S.W. 2753 Australia and J. L. ALBRIGHT, Department of Animal Sciences, Purdue University, West Lafayette, Indiana 47907.——A milking group of 16 Holstein-Friesian cows housed in a free stall barn at the Purdue University Dairy Center was observed over a period of four weeks in the spring of 1975.

Four of the cows were added to the previously stable group of twelve early in the observation period. The average age of the group was between six and seven years. It was part of a long-term urea feeding trial which keeps the group isolated over many years. The introduction of new cows to the group did not cause the upheaval expected. None of the introductions had been part of the group before.

All behavior which could possibly have social implications was recorded and an attempt was made to interpret it. Criteria for the more complex types of behavior were established with the aid of the literature reviewed and previous experience.

Both quantitative and qualitative data revealed a wide variety of behavior as probably having social implications. There were no readily distinguishable categories or social types into which the majority of the cows fell automatically. Some low ranking cows in the social order were far more aggressive than some cows that dominated them when interactions occurred.

Attention was also drawn to the complex and subtle nature of interactions and inter-cow communication in a group of cows forced to live at close quarters for long periods of time. Tolerance of conspecies in close proximity or actual body contact was notable.

Some of the variation from a predicted response was interpreted as being due either to a variation of intensity of the releasing stimulus or the intensity or manner in which the stimulus was received by the other cow. In other words, the intensity of a releasing situation depends on a number of variables in more than one animal.

One conclusion is that individual personalities of cows fall automatically into no watertight compartments, nor are they always instantly or easily recognized or deciphered. Nevertheless, their social disposition is seen as the clearest measure of their temperament or personality. Non-interaction itself is also seen as a temperament trait. The claim made by some researchers that the social order of a group of dairy cattle can be established after a full single day's observation cannot be sustained as far as this particular group of cows is concerned.

Diets of sympatric *Acris crepitans* and juvenile *Bufo woodhousei fowleri* in western Indiana. GEORGE M. LABANICK, Department of Zoology, Southern Illinois University, Carbondale, Illinois 62901 and RAYMOND A. SCHLUETER, Division of Science and Mathematics, University of Tampa, Tampa, Florida 33606.——Feeding competition between sympatric *Acris crepitans* and juvenile *Bufo woodhousei fowleri* was slight. *Bufo w. fowleri* exploited ground-dwelling ants whereas *A. crepitans* utilized a diversity of ground and above ground-dwelling prey.