

Collegiate Division

The following five articles are abstracts of papers presented at the Collegiate Division of the Tennessee Academy of Science in Oak Ridge on December 6, 1958.

STUDIES OF GENETIC FACTORS IN HOMEOSTASIS

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The purpose of these studies was to establish strain and sex differences in white blood cell counts of Albino mice under normal conditions and under such stress as can be shown by insulin injection.

Members of three generations of albino mice were used in the experiment. The mice were kept in the animal room under normal conditions and fed a standard laboratory diet prior to and during the studies. All experimental animals and "controls" were fasted overnight prior to the blood studies.

Normal leucocyte counts were taken, first in an attempt to establish a control pattern for albino mice, and second, to investigate the possibility of a genetic pattern in the normal leucocyte count. Blood was taken from the tip of the tail and diluted with one per cent hydrochloric acid and the total number of white cells counted. For our differential counts, Wright-Giemsa stain was applied to blood-smear slides and a differential leucocyte count was done on each animal at different times of the day. Our normal counts, which showed a ratio of three high to one low count in the F_2 generation indicates the possibility that the total leucocyte count is a genetic factor with high count being dominant over low count. With the number of animals used, no pattern of a differential count began to emerge. However, much more experimentation must be carried out before we can say that even the total leucocyte count is a genetic factor.

Next, we took counts on these same mice after they had been injected with insulin. Four-hundredths of a unit of U80 insulin was injected intraperitoneally into each mouse having a weight of approximately 28 grams, and counts were taken hourly for eight hours. Studies were made of the graphs which were constructed, and it was found that there was a definite increase in each total count, and then a gradual decrease. These graphs give us a good idea of the pattern which the counts make when this type of stress is applied to albino mice, but do not, as yet, reveal the possibility of "strain" differences.

We plan to continue our experiments and to perfect our procedure until definite findings begin to emerge.

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"CRAWFISH WITH ROCKS IN THEIR HEADS"

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The project is being carried out to attempt to discover the function of calcium deposits in the heads of soft-shelled crawfish. One hundred fifty-three crawfish have been caught and examined for evidence of the calcium deposits (gastroliths).

These crawfish were caught and data was recorded as to the number of hard shelled and soft shelled crawfish. The percentage of soft shelled specimens with gastroliths as compared with hard shelled specimens with gastroliths was tabulated. Other significant evidence as to color of the crawfish, size of the gastroliths as compared to body size, condition of the crawfish when varied sizes of gastroliths were found and position of the gastroliths was recorded.

Just before the crawfish sheds its hard exoskeleton, two gastroliths are formed just above the ventral body wall between the eyes and mouth. These gastroliths seem to be calcium carbonate and they attain their maximum size when the exoskeleton is shed. As the crawfish forms another hard shell the gastroliths seem to be dissolving. By the time the shell is again hard the gastroliths seem to be dissolved.

My hypothesis is that these gastroliths are used in hardening the shell. This is to be tested by keeping a soft shelled crawfish alive and X-raying it at intervals while the shell is being hardened.

OPERATION OF GENETIC FACTORS IN GROWTH AND DEVELOPMENT OF MICE

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An initial study of possible quantitative genetic factors yielded evidence for the following:

Albinos show hemophilic qualities and numerous post-natal deaths. The strain's mature weight of 22 gms. is reached within six months.

The litters of the Yellow Lethal mice have growth rates similar to the albino mice. Of 257 mice, 30 blacks and 119 heterozygotes reached maturity. The typical ratio for the yellow lethal strain at birth is two heterozygotes to one black. The average weight is 26 gms. with the growth curve leveling off at

the fifth month. Obesity is a constant problem with females after their first litter. The black offspring of the yellow lethal do not show blood pictures similar to the "normal" black stock.

The dwarf-breeders produce dwarfs in the ratio of one homozygous dwarf to two heterozygotes and one homozygous normal. Of seventeen litters, 21 of the dwarf mice survived weaning but only 13 lived over two months; whereas, 138 normal heterozygous litter mates survived weaning and 123 reached maturity. The normals and heterozygotes weigh 30 gms. within three and one-half months. The growth curve of the homozygous recessive dwarfs differs from that of "pituitary" or "pygmy" dwarfs. The achondroplastic condition (especially of the basicranium) suggests a different anomaly from the descriptions in the literature of the "pituitary" and "pygmy" dwarfs.

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THE EFFECT OF COLD STRESS ON PERIPHERAL LEUCOCYTE COUNT IN DWARF-CARRIER MICE

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Variation from normal total and differential white blood cell counts have been noted in "achondroplastic" dwarfs and in their response to "stress". This variation could possibly be noted also in those animals that carry this characteristic genotypically.

In hopes of finding a relation between the blood picture of the dwarf and a "heterozygote", the "heterozygotes" were exposed to a means of "stress" that would cause a change in count, in this case, cold temperature.

The mice, C-57 known and suspected heterozygotes, were placed in a refrigerator for a period of one hour and at a temperature of 2-7° C. After removal, their blood count was taken hourly to note the response to the "stress" by a variation in total WBC count and change in the differential count.

These counts, as may be observed in a composite graph of six mice, showed a slight rise after the first hour and then a continued drop during the next 3-4 hours, where it then leveled off. The "differential" showed an increase in number of polymorphonuclear white cells, with the rise in total WBC, or directly proportional.

In conclusion, it can be said that this is a probably abnormal picture as compared to other data; however, more data must

be obtained to compare or determine the degree of abnormality; e.g., response of known homozygote normal mice and to that of "achondroplastic" dwarfs.

Secondly, will other kinds of stress, i.e., electric shock, insulin shock, trauma, cause a similar response?

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FACTORS IN ADDITION TO EXPERIMENTAL "STRESS AGENTS" INFLUENCING THE LEUCOCYTE-COUNT IN ALBINO RATS

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The purpose of this work is to determine factors in addition to experimental "stress agents", such as insulin, that affect the leucocyte-count in albino rats. Such factors as weight and age, body temperature, sex, the reaction of the animal to handling, and differences in traumatic damage must be considered.

Rats used were an inbred strain of Sprague-Dawley animals. All animals, including treated "controls", were fasted for 24 hours prior to the experiment.

Blood was removed from the tail vein, as near the tip as possible. A total leukocyte count, using 1.0% HCl as diluting fluid, was taken as well as a differential count, using Wright-Giemsa stain in the earlier studies, and propylene glycol methylene blue-phloine method in the later ones. If insulin was to be used as a "stress agent", a normal count was taken from the animal before insulin was injected, based upon a dosage of 2.0 units of protamine zinc insulin per kilo. Counts were taken every hour on the hour for a period of eight hours. A series of counts was started at the same time every day so as to get consistent counts, and eliminate diurnal variations between animals. Rectal temperatures were taken in all animals at hourly intervals.

FINDINGS: The female is more erratic in its counts than is the male, both in normal counts and counts taken while the animal was under insulin stress. Due to this, we believe that the female is more susceptible to differences in handling and all external stress agents, than is the male.

We found that as the animal approaches insulin shock, there is generally a fall in total count, an increase in the lymphocyte count, and a decrease in the number of granulocytes. As the animal returns to normal, these counts tend to equalize.

The dosage of insulin must be close enough to lethal in its effects to approach shock, to obtain accurate and dependable counts. In our work we have injected all insulin intraperitoneally, at the same site and depth, and at body temperature, so that the insulin will be absorbed into the tissues at the same rate in all cases.

The total leucocyte count is to some degree correlated with the body temperature of the animal, the rectal temperature behaving inversely to total leucocyte count. We have also indications that the weight and age of the animal affects the total leucocyte count. There is an apparent correlation between total white count and total polymorph count, the polymorph count tending to go up in direct proportion to total count, the lymphocyte varying inversely.

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NEWS OF TENNESSEE SCIENCE

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"Extreme sensitivity of an immature stage of the mouse ovary to sterilization by irradiation," and Dr. Schwartz presented a paper entitled "Genetic studies on enzymes in maize endosperm."

Dr. W. L. Russell attended a Symposium on Molecular Genetics and Human Disease at the State University of New York Upstate Medical Center, in Syracuse, April 23-24. He presented a lecture entitled "Recent advances in genetics of mice."

Dr. Arthur C. Upton attended meetings of the Pathology Study Section of the U. S. Public Health Service, May 7-10.

The Biology Division Research Conference on "Enzyme Reaction Mechanisms" was held in Gatlinburg, Tennessee, April 1-4. Alvin M. Weinberg, Director of the Oak Ridge National Laboratory, presented the welcoming address.

Dr. Alexander Hollaender attended a meeting of the Biological Effects of Atomic Radiation Committee on genetic effects of radiation at the Rockefeller Institute in New York on May 13.

Jean Moutschen of Belgium has completed a one-year period of research in the Division under an ICA fellowship and has returned to the University of Liege, Belgium.

Dr. Howard I. Adler left May 29 to visit European laboratories. He will attend the Symposium on Immediate and Low Level Effects of Ionizing Radiations to be held at Venice, Italy, June 21-27, and present a paper at the conference entitled "Radiation behavior of a catalase negative bacterium." He will then attend discussions concerning mechanisms of radiation damage to living cells with scientific investigators in Italy, Denmark, and Sweden.

Dr. William Arnold will be at the Institute for Muscle Research, Woods Hole, Mass., from June 1 to September 7. Dr. Arnold will collaborate with Dr. Albert Szent-Gyorgyi during the summer.

Francis E. Kenney has joined the Enzymology Group. Dr. Kenney received a B.S. degree in biology from St. Michaels College, an M.S. degree zoology from the University of Notre Dame, and a Ph.D. degree in biochemistry from Johns Hopkins University. He has been associated with the Cornell University School of Medicine for the past two years.

RESEARCH GRANTS FOR UNDERGRADUATE STUDENTS

Dr. Arthur W. Jones, Associate Professor of Zoology at the University of Tennessee, is in charge of a new plan for qualified undergraduates majoring in science to participate in current research projects. The plan is being supported by a \$7,140 National Science Foundation grant. Participants will be paid \$1.50 an hour for up to 400 hours per participant. Applicants should be in the upper 20 per cent of their class, scholastically. The research includes current projects in botany, chemistry, and zoology. Students may apply by sending transcripts of their college work, along with statements of their interest in doing advanced work in appropriate fields. They should ask a professor in their field of interest to support their application by a confidential letter of recommendation. Applications or inquiries should be addressed to Dr. Arthur W. Jones, Department of Zoology and Entomology, The University of Tennessee, Knoxville.