

The wing bones of the normal and abnormal 29-day-old turkey embryos had a lower percentage of ash than the leg bones. This agrees with data for embryos of the domestic fowl of comparable age and perhaps indicates that this may be true for embryos of all gallinaceous species and perhaps all Aves. Since Weakley and Dustman³ found only slight differences between the percentage of ash in the humerus and femur of 5-week-old chicks, the wing bones presumably calcify more rapidly than the leg bones after the birds hatch.

Summary. A simple autosomal recessive lethal mutation that causes crowding together of the vertebrae with consequent shortening of the neck and body is described. The percentage of ash in the wing and leg bones was approximately the same as in the normal embryos, but the percentage of ash was less in the scapula, ilium, and ischium than in the corresponding bones of the normal embryos.

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Sex Differences in Pigment Content of Harderian Glands of Mice.

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Recent data^{1, 2} have shown that the Harderian glands of female mice of the C₃H strain show two characteristics that differ from those glands found in male mice of the same strain: (1) under normal light the exposed Harderian gland of a female, when stretched upon a microscope slide in a fresh or semidried state, shows a greater degree of "gray" pigmentation, and (2) under ultraviolet light (General Electric BH4) the glands from female mice tend to show a greater intensity of red fluorescence, thus indicating perhaps a higher concentration of porphyrins. (By differential solubilities, HCl numbers and spectroscopic data it has been shown that the porphyrin present in mouse Harderian glands is protoporphyrin.)

³ Weakley, C. E., Jr., and Dustman, R. B., *J. Agric. Res.*, 1939, **58**, 711; *W. Va. Bull.* No. 294, 40 pages, 1939.

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¹ Figge, F. H. J., Strong, L. C., Strong, L. C., Jr., and Shanbrom, A., *Cancer Research*, 1942, **2**, 335.

² Strong, L. C., and Figge, F. H. J., *Science*, 1941, **94**, 331.

In order to verify these observations and to extend the work, the Harderian glands of 468 mice of the C_3H strain have been examined; of these 226 have been from females, 242 from males. These data show that within an inbred strain it appears certain that two variables influence the degree of "gray" pigmentation under normal light. These are (1) sex, and (2) age. When the Harderian glands of litter mates are examined simultaneously it can readily be seen that the females show darker pigmentation than do males. The glands of both sexes appear to diminish in grayness with advancing age. It is also apparent² that at a given age the Harderian glands of female mice show more intense red fluorescence than do those from males of the same strain, thus verifying previous observations.

Nine female mice and 9 male mice at 50 days of age were examined simultaneously. The average body weight for the females was 14.66 g; that for the males 17.22 g. The average reading of red fluorescence for the females was 5.0; whereas for the males it was 3.33. Seventeen female mice at 75 days averaged 17.94 g; and 17 males weighed, on the average, 20.23 g. The average reading of fluorescence was: for the females 4.47; for the males 3.11. Twenty-one females at 250 days average 23.2 g; 22 males weighed, on the average, 28.57 g. Average reading of red fluorescence was for the females, 3.14; for the males, 3.08.

At 50 and 75 days (early sexual life) the females showed significantly greater "grayness" than did the males; whereas at 250 days there could be detected no sexual difference.

Glands for the 17 females and 17 males at 75 days of age were extracted separately for porphyrins. The 21 females and 22 males at 250 days were similarly employed. At every step in the following process the fluorescence for the material from the females was more intense than for the males at 75 days of age, but there appeared to be no difference at 250 days.

The glands were ground in sterile sand in a semi-dried condition. The resulting mixture was reddish-tan in color. Glacial acetic acid was added, 1 cc for each mouse (2 Harderian glands). The acid became deeply rose colored—more so for the female glands than for those of the males. Following addition of NaOH until the solution was nearly neutral to Congo red paper, an excess volume of ether was added. The ether was washed 3 times with distilled water, then 1% HCl was added 3 times and finally 10% HCl was added 3 times. All the fluorescent colored material came out from the ether into the 10% HCl solution (following a negative extrac-

tion with 1% HCl solution), thus indicating that the material extracted from the Harderian glands was protoporphyrin. Compared to a standard of coproporphyrin there was approximately 25% more protoporphyrin in the female Harderian gland than in a gland from a male of the same age (75 days) and strain (C_3H).[†] If a correction for body size be made, this sex difference is even more pronounced.

At 250 days there appeared to be no significant sex difference in the content of protoporphyrins as indicated by the intensity of fluorescence.

Conclusions. 1. The Harderian glands of female mice of C_3H strain at 75 days of life appear to differ in at least two ways from the Harderian glands of male mice of the same age and strain, as follows: (a) They appear to contain under normal light a greater degree of gray pigmentation; (b) they contain approximately 25% more protoporphyrins, in spite of the fact that the glands are smaller and the body weights are also smaller than in males. 2. These differences between sexes are more pronounced at 75 days of age than they are at 250 days.

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Experimental Transmission of St. Louis Encephalitis Virus by *Culex pipiens* Linnaeus.*

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Lumsden¹ and Casey and Broun,² on the basis of epidemiological investigations, suggested that the virus of St. Louis encephalitis is mosquito-borne. Mitamura and associates³ in Japan first reported successful transmission of St. Louis virus by a mosquito, *Culex*

[†] We are deeply indebted to Doctors C. P. Rhoads and K. Dobriner for their kindness in doing this quantitative determination for us.

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¹ Lumsden, L. L., unpublished official report, 1933.

² Casey, A. E., and Broun, G. O., *Science*, 1938, **88**, 450.

³ Mitamura, T., Yamada, S., Hazato, H., Mori, K., Hosoi, T., Kitaoka, M., Watanabe, S., Okubo, K., and Tenjin, S., *Tr. Jap. Path. Soc.*, 1937, **27**, 573.