each virus adsorbed in 30 minutes. This time has therefore been adopted in assays of both viruses.

The plaque assay of Mengovirus was compared with the assay in mice and the plaque assay of reovirus with the 50% infective endpoint titration in monkey kidney cultures. The plaque-forming units of both viruses are very similar in number to the infectious units in the ID_{50} assays. Reovirus plaques were read on the 9th day; corrected to the 14th day gives a titer of 3.3×10^8 PFU/ml. Thus it appears that plaque assays of both Mengovirus and reovirus-3 are as sensitive as ID_{50} assays and that repeated passage in L-cells does not result in loss of virulence for mice in the case of Mengovirus or for monkey kidney cells in the case of reovirus.

Summary. Reovirus-3 was passaged in L-cells where it grew and produced a cytopathic effect. A plaque assay in L-cells is described for this virus and compared with the plaque assay for Mengovirus.

Miss Joan Callender provided excellent technical assistance.

1. Sabin, A. B., *Science,* 1959, v130, 1387.

2. Pollock, M. E., Kenny, G. E., Syverton, J. T., PROC. SOC. EXP. BIOL. AND MED., 1960, v105, 10.

3. Eagle, H., *Science,* 1959, v130, 432.

4. Dick, G. W. A., *J. Immunol.,* 1949, v62, 575.

5. Ramcs-Alvarez, M., Sabin, A. B., *J. Am. Med. Assn.,* 1958, v167, 147.

6. Smith, J. D., Freeman, G., Vogt, M., Dulbecco, R., *Virology,* 1960, v12, 185.

Received May 25, 1961. P.S.E.B.M., 1961, v107.

Relationships of Testicular Cholesterol to Hormonal Activity and Behavior in the Starling.* (26717)

FREDERICK K. HILTON† (Introduced by William B. Atkinson)

The Johns Hopkins School of Hygiene and Public Health, Baltimore, Md., and Department of Anatomy, University of Louisville School of Medicine, Louisville, Ky.

Androgenic hormones are known to influence very dramatically behavior associated with courtship and breeding in many birds (1). In the British starling Bullough showed that these behaviors were greatly intensified during the period of most rapid growth of the testes(2). Witschi and Miller found that the color of the bill in both sexes of the starling is controlled by testosterone (3). As the gonads increase in mass the bill changes from black to yellow, and as the testes decrease in weight the bill returns to the black condition.

Cholesterol has been shown to be an important precursor in the biogenesis of the steroids of adrenals and testes(4,5,6). Marshall and others have observed histochemically changes which occur in the cholesterol-positive lipoids of avian testes during the annual cycylic activity(7). The present study conducted on starlings correlates the cyclic changes in mass of the testes with quantitative changes in concentration of cholesterol present in the organ. An hypothesis is advanced concerning the relationship of cholesterol levels to steroid hormone production. Correlations between behavioral activity of the starlings and general histological condition of the testes are also reported.

Material and methods. Starlings from a known stage in their behavioral cycle were killed with a shotgun. They were autopsied immediately and the testes were removed, blotted and weighed on a Roller Smith balance, and frozen in glass vials imbedded in dry ice. Cholesterol content of the testes was determined by the method of Knobil, *et al.*(8) and reported as total cholesterol since the free and esterified forms were not sepa-

* The data presented here were submitted to the School of Hygiene and Public Health, Johns Hopkins University, as a portion of a dissertation for the degree of Doctor of Science. This work was supported by N.I.H. Division of Research Grants, U.S.P.H.S.

† Present address, Univ. of Louisville, Ky.

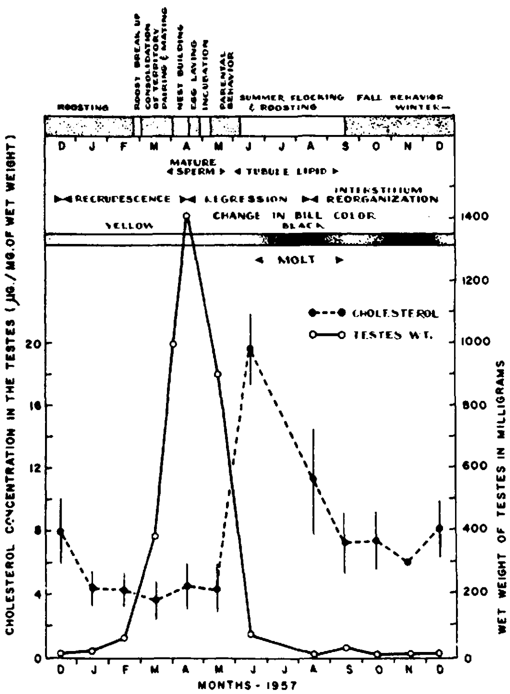

FIG. 1. Yearly cycles of behavior, weight and condition of testes, color of bill and concentration of cholesterol in testes of starlings.

rated. Tissue for histological study was preserved in Bouin's fluid.

Results. The results are summarized in Fig. 1 in which concentration of cholesterol in μg per mg of testes is plotted as the mean monthly value (dashed line) plus or minus 2 standard deviations. The variance was omitted for the November data since determinations from only 3 individuals were made during that month. A total of 110 starlings was used in the entire study and, with the exception of November, mean value for each month was based on analysis of between 8 and 10 individuals. The decrease observed in concentration of cholesterol between December and January is statistically significant ($P<.01$), as is the increase in concentration between May and June ($P<.01$). The marked decline between July and September is also statistically significant ($P<.01$). The solid line represents mean monthly weights of the testes used for the determinations. Across the top of the figure the time of molt, bill color change, and histological condition, as well as the various behavioral periods are indicated.

Discussion. While relatively little histological information for these particular starlings is available, a generalized pattern can be adapted from work on the starling testes by Bissonnette(9,10) and on other avian species by Marshall(11). It is apparent that certain correlations exist between the histological condition of the testes, cholesterol concentration in the testes, and annual cycle of behavioral and physiological changes in the starling.

The first important correlation to be considered is the decrease in cholesterol concentration which accompanies the very earliest onset of testicular recrudescence (December-January). The rapid decline in concentration is followed by a period of 4 months during which cholesterol concentration is constantly maintained at its lowest level. During this time the level of behavioral and physiological activities reaches a maximum and culminates in breeding. Presumably the hormonal secretions of the testes are primarily responsible for eliciting these responses. Rosenfeld(12) has shown that in the mammalian adrenal cholesterol concentration drops markedly when output of corticoids rises. If such is true of the testes of the starling this should be the time of maximum androgen production and/or utilization. It is suggested that the observed decline in concentration of cholesterol could be due to an increased use of this precursor molecule for the biogenesis of more testicular hormone.

A second correlation is observed during the latter phase of testicular regression in July and August when cholesterol concentration increases markedly at the time when cholesterol appears in the tubules. This might suggest non-use, altered or greatly reduced use of the cholesterol molecule in biosynthetic pathways. Lofts and Marshall (13) have shown in the spermatogentically active or recrudescing testes of the pigeon that the androgenic steroids androsterone and androstenedione are present in the interstitial tissue. In the regressing testes these androgenic hormones disappear and the lipid is found in the degenerating seminiferous tubules. From the intra-tubular lipid Lofts and Marshall have isolated progesterone and other progestins as well. It is also known that progesterone induces molt in some birds. Direct proof that in the starling the high concentration of cholesterol found in the regressing testes plays a role in production of progesterone or other steroid hormones is lacking. However, it is interesting to note the correlation that exists between onset of the molt and occurrence of the highest concentrations of cholesterol.

A third correlation is observed when the accumulation of cholesterol-positive material in the tubules slowly disappears and concentration decreases until it reaches a plateau during the start of fall territorial behavior. The amount of androgenic hormone present and/or utilized during the fall and winter months appears to be less than that available during the period of intensified spring activity, but more than that operative during the low ebb of behavior observed in July and August.

An hypothesis, based on the observed concentrations of cholesterol present throughout the annual testes cycle of the starling, can be illustrated by 3 phases: (a) The lowest cholesterol concentration, greatest hormone production and most active period of behavior are observed during courtship and breeding. (b) The highest cholesterol concentration, lowest hormone production, and period of least behavioral activity found at time of summer flocking. (c) Intermediate concentrations, moderate hormone production, and moderate behavioral activity are shown to accompany fall and winter territorialism.

Summary. Levels of cholesterol were determined in testes of starlings collected from all portions of the gonadal and behavioral cycle. The lowest concentration of cholesterol was observed during recrudescence of the testes and at the time of most active behavior. The highest concentration was observed during regression of the testes and at the period of lowest behavioral activity. Intermediate concentrations of cholesterol accompanied the moderate behavioral activity observed in fall and winter. An hypothesis relating cholesterol concentration, hormone production and behavioral activity is advanced.

1. Beach, F. A., *Hormones and Behavior,* Paul B. Hoeber, Inc., New York, 1948.

2. Bullough, W. S., *Philos. Trans. Roy. Soc.* (London), 1942, B 231, 165.

3. Witschi, E., Miller, R. A., *J. Exp. Zool.,* 1938, v79, 475.

4. Heard, R. D. H., Bligh, E. G., Cann, M. C., Jellinck, P. H., O'Donnell, V. J., Rao, B. G., Webb, J. L., *Recent Progr. Hormone Research,* 1956, v12, 45.

5. Hechter, O., Pincus, G., *Phys. Revs.,* 1954, v34, 459.

6. Roberts, S., Szego, C. M., *Ann. Rev. Biochem.,* 1955, v24, 543.

7. Marshall, A. J., Coombs, C. J. F., *Nature,* 1952, v169, 261.

8. Knobil, E., Hagney, M. G., Wilder, E. J., Briggs, F. N., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 48.

9. Bissonnette, T. H., *Am. J. Anat.,* 1930, v45, 289.

10. Bissonnette, T. H., Wadlund, A. P. R., *ibid.,* 1930, v45, 403.

11. Marshall, A. J., *Wilson Bull.,* 1951, v63, 238.

12. Rosenfeld, G., *Endocrinology,* 1955, v56, 649.

13. Lofts, B., Marshall, A. J., *J. Endocrin.,* 1959, v19, 16.

Received April 28, 1961. P.S.E.B.M., 1961, v107.