Bioassay of Human Tissues for Ecdysone.* (27633)

WALTER J. BURDETTE
Department of Surgery and Laboratory of Clinical Biology, University of Utah College of Medicine, Salt Lake City

Brain(1), corpus allatum(2), and prothoracic gland(3,4,5) of insects(6,7) have yielded respective active hormones on bioassay. Recently Kobayashi and co-workers(8) found cholesterol to be an active component in the brain of *Bombyx*. Schmialek(9) isolated farnesol and, with Wigglesworth(10), showed it to act in a manner similar to juvenile hormone (neotenin). Extracts of tissues from diverse biological sources have yielded positive bioassays for the presence of juvenile hormone(11). Since recent investigations(12,13) have demonstrated response of mammalian cells *in vivo* and *in vitro* to farnesol and ecdysone, whether ecdysone occurs in vertebrates (particularly human tissues) is also of interest.

Fortunately an excellent method of bioassay, the *Calliphora* test(14), is available for assessing the possible content of ecdysone in biological material. Fresh cadaveric tissue was obtained at autopsy and extracted by the same method yielding active material from *Bombyx* and *Drosophila* at appropriate stages of development(3,15). This was then tested for activity by injecting the posterior larval segment of ligated *Calliphora* in which the anterior segment alone pupated. The results are recorded in Table I.

No activity was found in any of the human tissues tested, although extraction of *Bombyx* chrysalises by the same process yielded active material. Large quantities of liver, brain, skeletal muscle, small intestine, and lung were used. In addition to the yields listed, quantities of testis, cardiac muscle, stomach, adrenal, pancreas, thyroid, and uterus of 500 g or less also failed to yield positive bioassays. Therefore, no evidence was obtained that ecdysone is contained in the human tissues tested or the amount is so small that the sensitive methods used failed to detect it.

The technical assistance of Mr. Lloyd Paul, Miss Gale Christensen, and Mr. Nick Wiser is acknowledged with appreciation.

1. Kobayashi, M., Kirimura, J., *Nature,* 1958, v181, 1217.
2. Williams, C. M., *ibid.,* 1956, v178, 212.
3. Burdette, W. J., *Science,* 1962, v135, 430.
4. Fukuda, S., *J. Fac. Sci. Univ. Tokyo,* 1944, vIV 6, 477.
5. Williams, C. M., *Biol. Bull.,* 1952, v103, 120.
6. Van Der Kloot, W. G., *ibid.,* 1955, v109, 276.
7. Wigglesworth, V. B., *Symposia of Exp. Biol.,* 1957, No. XI, 204.
8. Kobayashi, M., Kirimura, J., Saito, M., *Mushi,* 1962, v36, 85.

TABLE I. Bioassays of Human Tissue for Ecdysone.

Tissue	Wt (g) Wet sample	Wt (g) Extract	No. of assays	Highest % pupation (weighted)*	Activity
Bombyx	2000	20	3	83.0	+†
Homo					
Liver	7000	21.3	7	5.0	—
Brain	6000	8.6	8	18.8	—
Skeletal muscle	6000	137.6	7	25.0	—
Small intestine	5162	155.1	2	29.0	—
Lung	3618	63.4	9	23.8	—
Colon	2451	104.5	3	32.5	—
Kidney	1140	22.6	4	10.0	—
Spleen	703	66.0	4	0.0	—

* >50%:Positive.

† 1.1 γ = 1 Calliphora unit.

* Supported by a grant from Nat. Inst. Health, U.S.P.H.S.

9. Schmialek, V. P., *Z. Naturforsch.*, 1961, v16, b, 461.

10. Wigglesworth, V. B., *J. Ins. Physiol.*, 1961, v7, 73.

11. Gilbert, L. I., Schneiderman, H. A., *Anat. Rec.*, 1958, v131, 557.

12. Burdette, W. J., *Acta*, 1962, in press.

13. Burdette, W. J., Richards, R. C., *Nature*, 1961, v189, 666.

14. Becker, E., Plagge, E., *Biol. Zentr.*, 1939, v59, 326.

15. Butenandt, A., Karlson, P., *Z. Naturforsch.*, 1954, v9, b, 389.

Received June 6, 1962. P.S.E.B.M., 1962, v110.

The Parenchyma/Collagen Ratio in Normal and Hypertrophic Rat Kidneys. (27634)

IRMGARD MONTFORT AND RUY PÉREZ-TAMAYO (Introduced by A. A. Liebow)
Unidad de Patología, Facultad de Medicina, Universidad Nacional Autónoma de México, Hospital General, México, D. F.

Our previous work has shown that the parenchyma/collagen (P/C) ratio tends to remain constant in rat and human uteri during gestation and post-partum involution(1). In normal and hypertrophic human hearts the muscle/collagen ratio has been found to remain constant despite variations in age, sex, weight, and underlying disease(2). In view of these findings, it was considered pertinent to study the P/C ratio in other organs which would lend themselves to this type of analysis. MacKay *et al.*(3) and Addis and Lew(4) observed that after unilateral nephrectomy in the rat the remaining kidney would undergo hypertrophy for 20 days, after which weight increase continued only at the rate expected for general body growth. The highest degree of hypertrophy was 70% of normal kidney weight. The present work has explored the P/C ratio in normal and hypertrophic rat kidneys.

Material and methods. Twenty rats of Wistar strain weighing 79 to 159 g were kept in metal cages, fed a standard diet* and water *ad libitum*, and weighed daily throughout the experiment. After one week of observation they were lightly anesthetized with ether and a left nephrectomy was performed through a small lumbar incision. Groups of 10 rats were sacrificed with excess ether anesthesia at 10 and 20 days after unilateral nephrectomy, and the remaining kidney obtained. All kidneys were treated as follows: immediately after removal they were cleansed of fat, decapsulated and weighed. Total water content was determined by drying in oven at 100°C until constant weight, total protein by the Kjeldahl method, and total collagen by the technic of Neuman and Logan(5) as modified by Martin and Axelrod(6). The difference between total protein and total collagen was considered an adequate quantitative index of parenchyma, since the contribution of other proteins (elastin, smooth muscle) was probably very small.

To distinguish increase in kidney weight due to hypertrophy from that accompanying general body growth during the 20 days of the experiment, the relation of kidney weight to body weight at time of unilateral nephrectomy was expressed as a regression line(3,4, 7,8). Such regression line was extrapolated to the highest body weight reached at the end of experiment, and weights of the hypertrophic kidneys obtained at 10 and 20 days after unilateral nephrectomy were plotted in the same chart. The difference between the theoretical kidney weight for a given body weight, and the actual value encountered at time of sacrifice was the measure of hypertrophy. The regression coefficient for the correlation between kidney weight and body weight was tested for significance by dividing the regression coefficient by the standard error of the regression coefficient. This resulted

* Purina rat pellets.