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**Assay of Adrenal Cortical Extract.**

R. J. SCHACHTER AND M. O. BEBEE, JR. (Introduced by A. J. Carlson.)

*From the Department of Physiology, University of Chicago.*

The effectiveness of adrenal cortical extracts can at present only be determined by their ability to maintain the lives of completely adrenalectomized animals. The animals which have been used in assaying adrenal cortical extracts are the cat, dog and rat. The guinea pig, which makes an excellent animal for bioassay of adrenal cortical extract, has been neglected because of the anatomical difficulty in extirpating the right adrenal gland.

This paper reports a simple technic for removing the adrenal glands in the guinea pig, and discusses the advantages which are gained by the use of the guinea pig instead of the cat, dog and rat.

To our knowledge, Simmons and Whitehead<sup>1</sup> are the only authors who describe a technic for the adrenalectomy in the guinea pig. By their method a great deal of shock is caused to the animal because they crush the penultimate rib and draw the kidney and the adrenal out through the wound. This shock is avoided by the following technic:\*

1. Animals which have been fasted for one day are anesthetized with ether and turned on their left side. The right side is shaved, and the following description is for a right adrenalectomy.

2. A straight line incision approximately one inch long is made parallel to and immediately under the last rib. The incision extends about a half inch medial to the end of the last rib.

3. The liver is packed up, and the intestine medially with one pack each.

4. With the finger retracting the kidney caudalwards, the peritoneum, covering the ventral surface of the kidney and the adrenal gland, is torn with forceps to free the caudad margin of the adrenal.

5. The lateral and craniad margins of the adrenal gland are freed by cautiously tearing the ligaments joining the gland laterally to the body wall, and cranially to the diaphragm.

6. The posterior portion of the adrenal is cleared by turning over the freed craniad portion, and then removing the connective tissue from the underlying body wall. The gland is now only attached to the vena cava.

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<sup>1</sup> Simmons, H. T., and Whitehead, R., *J. Physiol.*, 1936, **88**, 235.

\* The surgery is cinema recorded.

7. The ligament, lying between the body wall, the vena cava, and the adrenal gland, is put under tension by downward traction on the kidney. The ligament is picked up by forceps in the right hand, then while traction on the kidney is released, the ligament is picked up with another pair of forceps.

8. Anchoring the lateral portion of the ligament with the forceps in the left hand, continued vertical traction by the forceps in the right hand is exerted until the ligament is torn. (This method of freeing the gland is the essential feature of the operation. The connective tissue that is being torn must be visible at all times, otherwise the vena cava is endangered.)

9. The caudad pole of the gland may now be picked up by the capsule, and rotated so that the underlying vena cava is visible. Upward tension stretches the fibers covering the gland and the vena cava, thus the gland may be removed from the vena cava, leaving the gland attached only to the adrenal vein.

10. The adrenal vein is clamped for about 30 seconds with one pair of forceps, with another pair of forceps placed between the clamping forceps and the gland enough traction is exerted to remove the gland from the body.

11. The body wall is closed by suture and the skin is closed by clips.

Since the adrenal gland of the guinea pig is very fragile, care is taken not to handle the gland with any instrument.

The left adrenal gland is removed in similar fashion, but less care is required, since the left adrenal gland does not lie on the vena cava.

In this work 50 guinea pigs were used; 20 were used for controls and 30 were treated with adrenal cortical extract. The control animals died of adrenal insufficiency at an average of 4.5 days (extremes 3 to 7 days).

The assay of the extract is as follows: The right adrenal of a 400-600 g guinea pig was removed, and 2 weeks later the left adrenal was removed. The day following the removal of the second adrenal, enough extract was injected for a period of 10 to 12 days, so that the pig gained weight and was in an apparent normal state. Following the withdrawal of the extract, the pig died of adrenal insufficiency within 3 to 4 days. Necropsy never revealed any accessory adrenal cortical tissue.

A unit is considered that amount of extract which, when 1 cc is injected daily, will maintain 1 kg of guinea pig in an apparently normal state for 10 days. Usually 1 cc equals 45 g of fresh whole adrenal tissue.

The advantage is that, once an extract is assayed for one guinea pig, it applies to most guinea pigs. This is not true of dogs.

Although cats and dogs have practically no accessory adrenal cortical tissue, they do not lend themselves for bioassay as well as does the guinea pig, because (1) a great many cats following surgery, even of only one adrenal, refuse to take their food, and die of starvation or have to be fed by stomach tube; (2) dogs, because of their size, require large quantities of extract.

The advisability of using the rat in this assay work is very questionable, because there is considerable controversy as to the survival period of the rat following adrenalectomy. One group of workers<sup>2-7, 13</sup> claim that the majority of rats indefinitely survive adrenalectomy, while another group<sup>8, 9, 10</sup> find that the majority of rats will survive adrenalectomy for only a few days. The most recent trend seems to be that the survival period of adrenalectomized rats depends on their diet<sup>11-16</sup> and environmental condition.<sup>14, 17-20</sup>

Another disadvantage of the rat is that it requires very large doses of extract to keep it alive. Thus, Cartland and Kuizenga<sup>21</sup> found that the rat requires 22 times as much extract per unit weight as does the dog, while D'Amour and Funk<sup>22</sup> found that a 50 g rat requires as much extract as does a 15 kg dog.

<sup>2</sup> Scott, W. J., *J. Exp. Med.*, 1923, **38**, 543.

<sup>3</sup> Jaffe, H. L., *J. Exp. Med.*, 1923, **38**, 107.

<sup>4</sup> Lewis, J. T., *Am. J. Physiol.*, 1923, **64**, 503.

<sup>5</sup> Rogoff, J. M., and DeNecker, J., *J. Pharm. Exp. Therap.*, 1925, **26**, 243.

<sup>6</sup> Wyman, L. C., *Am. J. Physiol.*, 1928, **86**, 528.

<sup>7</sup> Marmorston-Gottesman, J., and Perla, D., *J. Exp. Med.*, 1932, **55**, 109.

<sup>8</sup> Pencharz, R. I., Olmstead, J. M. D., and Giragossintz, G., *Science*, 1931, **72**, 175.

<sup>9</sup> Freed, S. C., Brownfield, B., and Evans, H. M., *PROC. SOC. EXP. BIOL. AND MED.*, 1931, **29**, 1.

<sup>10</sup> Martin, S. J., *Am. J. Physiol.*, 1932, **100**, 180.

<sup>11</sup> Rubin, M. I., and Krick, E., *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **31**, 228.

<sup>12</sup> Kutz, R. L., McKeown, T., and Selye, H., *PROC. SOC. EXP. BIOL. AND MED.*, 1934, **32**, 331.

<sup>13</sup> Gaunt, R., Tobin, C. E., and Gaunt, J. H., *Am. J. Physiol.*, 1935, **111**, 321.

<sup>14</sup> Agate, F. J., and Zwemmer, R. L., *Am. J. Physiol.*, 1935, **111**, 1.

<sup>15</sup> Cleghorn, R. A., Cleghorn, S. M. M., Forster, M. G., and McVicar, G. A., *J. Physiol.*, 1936, **86**, 229.

<sup>16</sup> Swann, H. G., *Am. J. Physiol.*, 1937, **118**, 798.

<sup>17</sup> Wyman, L. C., and TumSuden, C., *Am. J. Physiol.*, 1929, **89**, 362.

<sup>18</sup> Hartman, F. A., Brownell, K. A., and Crosby, A. A., *Am. J. Physiol.*, 1931, **98**, 674.

<sup>19</sup> Firor, W. M., and Grollman, A., *Am. J. Physiol.*, 1933, **103**, 686.

<sup>20</sup> Weiser, R. S., and Morris, E. B., *Endocrinol.*, 1936, **20**, 556.

<sup>21</sup> Cartland, G. F., and Kuizenga, M. H., *Am. J. Physiol.*, 1936, **117**, 678.

<sup>22</sup> D'Amour, F. E., and Funk, D., *J. Pharm. and Exp. Therap.*, 1938, **62**, 307.

The disadvantages of the cat, dog and rat as assay animals for adrenal cortical extracts may be circumvented by the use of the guinea pig, because the guinea pig has no accessory adrenal cortical tissue, the surgery is comparatively simple, and the adrenalectomized guinea pig requires comparatively little extract to maintain it in an apparent normal state.

*Summary.* The merits of the cat, dog, rat and guinea pig as assay animals for adrenal cortical extracts is discussed. The guinea pig is found to be the most satisfactory animal because (1) it is easy to operate and handle; (2) it has no accessory adrenal cortical tissue, and (3) it requires small amounts of extract to maintain it in an apparently normal state.

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### Modification of Sexual Development in the Opossum by Sex Hormones.\*

CARL R. MOORE.

*From Hull Zoological Laboratory, The University of Chicago.*

Sex hormone application modifies to some extent the embryonic development of the reproductive system in reptiles, birds and mammals.<sup>1-7</sup> Immature pouch young of marsupials might be expected

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<sup>1</sup> Kozelka, A. W., and Gallagher, T. F., *PROC. SOC. EXP. BIOL. AND MED.*, 1934, **31**, 1143.

<sup>2</sup> Willier, B. H., Gallagher, T. F., and Koch, F. C., *Proc. Nat. Acad. Sci.*, 1935, **21**, 625; *Physiol. Zool.*, 1937, **10**, 101.

<sup>3</sup> Wolfe, E., and Ginglinger, A., *Arch. d'anat., d'Hist., et d'Emb.*, 1935, **20**, 219.

<sup>4</sup> Dantchakoff, V., *Bull. Biol.*, 1936, **70**, 241; 1937, **71**, 269.

<sup>5</sup> Greene, R. R., Burrill, M. W., and Ivy, A. C., *Science*, 1937, **86**, 200; 1938, **87**, 396; **88**, 130.

<sup>6</sup> Hamilton, J. B., and Gardner, W. U., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **37**, 570.

<sup>7</sup> Raynaud, A., *Bull. Biol.*, 1938, **72**, 297.