25. Barker, S. B., Summerson, W. H., *ibid.*, 1941, v138, 535.

26. Fisher, R. A., *Statistical Methods for Research Workers,* Oliver and Boyd, Edinburgh, 1950.

27. Madison, L. L., Unger, R. H., *J. Clin. Invest.*, 1958, v37, 631.

28. Root, M. A., *J. Pharmacol. Exp. Ther.*, 1957, v119, 468.

29. Henry, W. L., Kim, J. H., Hall, A. S., *Am. J. Physiol.*, 1958, v192, 514.

30. Holt, v. C., Kracht, J., Kröner, B., Holt, v. L., *Schweiz. Med. Wschr.*, 1956, v86, 1123.

Received July 28, 1958. P.S.E.B.M., 1958, v99.

## Progestational Activity of Certain Steroid-17-Spirolactones. (24380)

ROY HERTZ AND WILLIAM W. TULLNER

*Endocrinology Branch, National Cancer Inst., N.I.H., Bethesda, Md.*

The structural requirements for estrogenic activity have long been known to be highly non-specific. Nevertheless, it has been previously considered that progestational activity depends upon highly specific structural relationships. More recently, a number of steroids which represent major structural deviations from the progesterone molecule have been shown to actually exceed progesterone itself in biological potency(1-4). Accordingly, in assessing the progestational potency of a wide variety of steroidal agents, we found that the synthetic steroid 3-(3-oxo-17$\beta$-hydroxy-4-androsten-17$\alpha$-yl) propionic acid $\gamma$-lactone (I) and its 19-nor analogue (II) possess substantial progestational activity as determined by a previously described modification of the Clauberg assay(1,2). In these assays we employed 2 samples of compound II and one sample of compound I. Each sample has been checked for chemical homogeneity by melting point determination and chromatographic analysis.*†

TABLE I. Progestational Activity of Steroid-17-Spirolactones.

| Series | Total dose (mg) | Progestational proliferation* Compound II | Progesterone |
|---|---|---|---|
| A | 5 † | +4, +4 | |
| | 1 † | +3, +1, +2 | |
| | .5 | | +4, +3 |
| B | 1.0 | +2, +2, +2 | |
| | .5 | +2, +3, +1 | +4, +3, +1 |
| | .25 | +1, +1, +1 | +4 |
| C | 1.0‡ | +3, +2, +3 | |
| | .5 | +2, +2 | |
| | .25 | +1, +1 | +2, +2, +1 |
| | | Compound I | |
| D | 10 | +3, +2, +3 | |
| | 5 | +2, +2, +2 | |
| | 1 | 0, 0 | |
| | .5 | | +3, +3, +4 |

* Each number represents one animal.

† Administered as a suspension. Sesame oil solution given in all other cases.

‡ Oral admin.: Compound II = 19-nor-3-(3-oxo-17$\beta$-hydroxy-4-androsten-17$\alpha$-yl) propionic acid $\gamma$ lactone. Compound I = 3-(3-oxo-17$\beta$-hydroxy-4-androsten-17$\alpha$-yl) propionic acid $\gamma$ lactone.

*Results.* The data presented in Table I permit only an approximate comparison of the potency of these spirolactones with that of other parenterally or orally active progestogens. However, it may be estimated that the 19-nor-analogue (II) is about half as potent by subcutaneous administration as progesterone. Similarly, I is about 1/10th as active as progesterone when each is given subcutaneously. Moreover, II is approximately as active as 19-nor ethinyl testosterone when each is given orally(2).

It is of special interest that these steroid-17-spirolactones have been shown to antagonize the sodium-retaining effects of desoxycorticosterone and of aldosterone in rat and man(5,6). Desoxycorticosterone is itself a weak progestogen. In addition, progesterone has been shown to produce natriuresis in rat and man, a metabolic effect quite comparable to that seen with the steroid-17-spirolactones (5,7). Hence we are confronted with an apparent paradox in that progestational action

* We are indebted to Dr. M. B. Lipsett for these chromatographic analyses.

† We wish to thank Dr. Victor Drill, Searle & Co., for providing the steroid-17-spirolactones used.

is shared by desoxycorticosterone, the steroid-17-spirolactones, and progesterone, whereas the spirolactones and progesterone actively antagonize the sodium-retaining action of desoxycorticosterone. These discrepancies may follow naturally from the fact that the biological effects of these steroids are mediated on the one hand through the endometrium and on the other, through the renal tubular apparatus. In any case, the apparent overlap between mineralocorticoid, anti-mineralocorticoid and progestational action of these several compounds should provide further understanding of salt and water metabolism in normal and abnormal pregnancy.

*Summary.* The steroid-17-spirolactones (I and II) have been shown to have progestational activity in the estrogen-primed immature rabbit.

1. Tullner, W. W., Hertz, R., *Endocrinology,* 1953, v52, 359.
2. Hertz, R., Tullner, W. W., Raffelt, E., *ibid.,* 1954, v54, 228.
3. Ehrenstein, M., Barber, G. W., Hertz, R., *ibid.,* 1957, v60, 681.
4. Rock, J., Pincus, G., Garcia, C. R., *Science,* 1956, v124, 891.
5. Kagawa, C. M., Cella, J. A., Van Arman, C. G., *ibid.,* 1957, v126, 1015.
6. Liddle, G. W., *ibid.,* 1957, v126, 1016.
7. Landau, R. L., Bergenstal, D. M., Lugibihl, K., Kascht, M. E., *J. Clin. Endocrinol. and Metab.,* 1955, v15, 1194.

Received August 4, 1958. P.S.E.B.M., 1958, v99.

## The Preparation and Use of Formalinized Erythrocytes with Attached Antigens or Haptens to Titrate Antibodies. (24381)

JOSEPH S. INGRAHAM (Introduced by E. W. Shrigley)
*Dept. of Microbiology, Indiana University Medical Center, Indianapolis, Ind.*

Red blood cells bearing soluble antigens or haptens attached to the surface have proven a sensitive reagent for detection of antibodies (1,2,3) or antigens(4). For agglutination titrations there are advantages in the use of red cells which have been stabilized by treatment with formalin. When prepared according to published methods(5,6), the cells become very adherent and require prolonged violent homogenization to obtain suspensions for use. The resulting suspensions are reported to agglutinate spontaneously in presence of traces of formaldehyde(5,6), and prolonged washing followed by treatment with bisulfite and dialysis has been recommended to free the cells from loosely bound formaldehyde(6). Cells treated with formaldehyde by the method described below can be dispersed by manual shaking and are not agglutinated by whatever traces of formaldehyde remain after several washes with saline. Some cells have retained their original serological activity after storage in dilute formalin in refrigerator for 15 months.

*Methods. Preparation of cells.** Human type O red cells were used within 2 to 3 weeks after drawing into acid-citrate-dextrose solution. The cells were washed 4 times with about 10 volumes of 0.85% sodium chloride containing 0.005 molar sodium phosphate pH 7.3 (hereafter referred to as saline) and 0.5% dextrose. A 50% suspension of washed cells was made in saline, chilled to 1-5°C and poured into 4 volumes of cold 10% formalin-saline solution. This solution was prepared by diluting 1 volume of neutralized reagent formaldehyde solution† with 3 volumes of 1⅓ times concentrated saline. A final suspension containing 10% red cells and 8% formaldehyde in saline at pH 7 to 7.3 was thus obtained. The suspension was stored at 1 to 3°C in a stoppered flask and the cells resuspended once each day for 10 days by shaking and swirling by hand. Cells treated

* Human cells of various types were obtained through generous cooperation of Miss Narcissa Hocker of Indiana University Medical Center Blood Bank.

† J. T. Baker Co. reagent 37% HCHO containing 11.9% methanol and neutralized to pH 6 to 7 using 1 molar NaOH.