Addendum to the Note of Williams and Watson

By James W. McBain

I do not consider that the note by Williams and Watson calls for any detailed comment. The two sentences published by McBain and O'Sullivan, one sentence in a preliminary communication and the other single sentence in a footnote in a paper of eleven printed pages entitled "The Development of the Air-Driven Spinning Top as a Transparent Ultracentrifuge," still appear to be ample notice of the physical phenomenon that was observed, on November 15, 1934, with the old circular cell whose use was discontinued that year. The cell contained pure water and a crystal of mercuric chloride. Sufficient of the latter dissolved in the lower portion of the cell and then through an accident of convection currents, the absorbing material ($HgCl_2$) was sedimented downward again with a fairly sharp boundary as shown by the photographs and microphotometer photographs, moving 0.102 cm. in 8400 seconds at 108,600 r. p. m. with an average radius of 1.054 cm., giving an observed $S = MD \times (1 - V)/RT = 0.890 \times 10^{-13}$, which, as a mere scientific curiosity, agreed with the value predicted. The precise agreement is of course fortuitous and should not have been expressed to three significant figures, as is obvious to anyone who has any knowledge of an ultracentrifuge.

Professor Williams has acknowledged Dr. O'Sullivan's statement that the sedimentation force was 138,000 times gravity, but Williams and Watson prefer to state "it is concluded that it was not over 350,000 times gravity."

McBain and O'Sullivan stated that this result was an accident and that in the ordinary course, where convection does not occur, a higher order of magnitude of centrifugal force than that in any existing ultracentrifuge anywhere today would be required to obtain sedimentation velocities of such small molecules regularly.

It is entirely a matter of individual opinion as to whether any significance whatsoever attaches to this photographed and microphotometered observation, and Williams and Watson are convinced on the basis of their experience that none does. We still think that it did offer an actual semi-quantitative measurement.

As regards the fourth paragraph of the note of Williams and Watson devoted to discussing the state of development of our air-driven ultracentrifuge as described at the April, 1935, meeting of the Society, it is only necessary to say that our statements were carefully made and stand accordingly as referring to the now superseded models as developed at that time; and that the appreciable modifications and the very simple temperature control in the later model referred to in the footnote by H. J. Fouts inserted at the end of that paper in the final proofs have resulted in uniform success in sedimenting smaller protein molecules such as carbon monoxide hemoglobin—as was, for example, demonstrated at the Western Protein Conference, June 6, 1936. There appears now to be no reason why any sedimentation velocity or equilibrium observable in the Svedberg ultracentrifuges, or the equally good air-driven models now developed, for example, at the Rockefeller Institute for Medical Research [see for example *J. Exptl. Medicine*, **64,** 39 (1936)], or the model supplied by the Sharples Specialty Company of Philadelphia should not also be capable of quantitative study in the McBain–O'Sullivan–Fouts transparent ultracentrifuge. All parts of this cell are easily kept at any desired *constant* measured temperature within 0.02°. All forms of the air-driven ultracentrifuge appear to be in process of still further rapid improvement.

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Chemical Nature of δ-Follicular Hormone

By O. Wintersteiner, E. Schwenk, H. Hirschmann and B. Whitman

In 1932 Schwenk and Hildebrandt[1] reported the isolation from the urine of pregnant mares of a new oestrogenic substance, isomeric with oestrone (theelin), which they designated δ-follicular hormone. Wintersteiner, Schwenk and Whitman[2] subsequently showed that this substance (m. p. 209°) was not a ketone, but a dihydroxy compound. Recently larger amounts of crude phenolic fractions from mares' urine were separated in the laboratories of the Schering Corporation into ketones and alcohols. The alcoholic portion was worked up for δ-hormone at Columbia University. Our present experience with material of this type makes it appear doubtful whether the earlier preparations represented a chemical individual,

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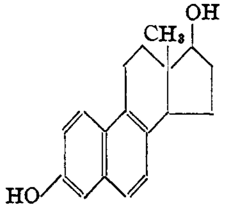
(1) Schwenk and Hildebrandt, *Naturw.*, **20**, 658 (1932).

(2) Wintersteiner, Schwenk and Whitman, *Proc. Soc. Exptl. Biol. Med.*, **32**, 1087 (1935).

a possibility discussed in the first publication. Fractional crystallization yielded an apparently homogeneous product which did not change its melting point (226° corr.) on repeated recrystallization from various solvents. However, the analytical composition and specific rotation varied with different preparations ($[\alpha]_D$ +31 to +37°). The preponderant constituent of these mixtures appears to be a molecular compound consisting of two components. Only one component forms a picrate, and this property as well as its stronger acidic character enabled us to isolate this component in pure form.

The new compound melts at 215–217° and has the composition $C_{18}H_{20}O_2$ (calcd.: C, 80.55; H, 7.52; found: C, 80.53, 80.47; H, 7.61, 7.52); $[\alpha]^{25}_D$ −4.7° (0.7% in dioxane). A di-*p*-nitrobenzoate (m. p. 250–252° corr.) and a monobenzoate (m. p. 203–205° corr.) have been prepared. The compound gives the color reactions originally attributed to the δ-follicular hormone.[1,3] Its absorption spectrum coincides with that of equilenin. Our measurements on equilenin reveal two new bands in addition to those found by Dirscherl and Hanusch,[4] namely, at 2310 Å., log ε 4.78, and at 2920 Å., log ε 3.58.

The monobenzoate was oxidized with chromic acid to a ketone which gave no depression of its melting point (223° corr.) when mixed with equilenin benzoate. The dihydroxy compound (m. p. 217°) is therefore dihydroequilenin.



Contrary to the rule, established for other oestrogenic compounds, that reduction of the C_{17} keto group to carbinol enhances the physiological activity, the dihydroequilenin isolated from urine possesses only about one-half of the potency of equilenin. On the other hand, David[5] found that the oily product which he obtained by reduction of equilenin with sodium was about three times as potent as equilenin. The possibility that our diol differs from the potent oestrogenic diols, obtained by reduction, in the configuration of the C_{17} hydroxyl group will be investigated. Whether the high potency originally reported for the δ-hormone resides in the other, as yet unidentified, component of the molecular compound (m. p. 226°) or in other diols present in the impure preparations, remains to be determined.

We wish to express our sincere thanks to Dr. A. Girard of Paris for sending us samples of equilenin and its benzoate for comparison.

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[3] Schwenk and Hildebrandt, *Biochem. Z.*, **259**, 240 (1933).

[4] Dirscherl and Hanusch, *Z. physiol. Chem.*, **233**, 13 (1935).

[5] David, *Acta brevia Neerl.*, **4**, 63 (1934),

Phenylmercuric Fluoride

BY GEORGE F. WRIGHT[1]

The recent discovery[2] of mercuric fluoride as a new fluorinating agent recalls the investigation in this Laboratory of phenylmercuric fluoride. This preparation was incidental in the study of the relative strengths of C–Hg and Hg–X bonds in organomercuric halides. According to our knowledge of organomercurials the tendency toward the dissociation reaction $2RHgX \rightleftarrows R_2Hg + HgX_2$ decreases in the order HgI > HgBr > HgCl. It was hoped that the introduction of fluorine would so stabilize the Hg–X bond as to favor the primary reaction $RHgX \longrightarrow R— + HgX$ which, it is considered, accounts for the interconversion possible with compounds like furyl and thienylmercuric halides containing more reactive, though unfortunately less stable, radicals.[3] The compound did not, however, fulfil these expectations. Instead of reacting with acetyl chloride to give acetophenone, acetyl fluoride was formed as easily as from mercuric fluoride.[2] Likewise when the compound was pyrolyzed at a temperature lower than that required to decompose diphenylmercury, only the latter substance, and no diphenyl, was produced.

In connection with the assertion of Henne and Midgley that pure mercuric fluoride cannot be prepared by treating mercuric oxide with aqueous hydrofluoric acid it may be significant that treatment of such a solution with phenyldiazonium fluoride, and subsequent treatment with copper, produced no trace of phenylmercuric fluoride.

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[1] National Research Fellow in Chemistry.

[2] Henne and Midgley, THIS JOURNAL, **58**, 884 (1936).

[3] Steinkopf and Bauermeister, *Ann.*, **403**, 50 (1914); Gilman and Wright, THIS JOURNAL, **55**, 3302 (1933).