through the fact that the galactosan, but not the glucosan, condenses easily with acetone. Acetone-D-galactosan yields D-galactosan (m. p. 223–224 cor., $[\alpha]^{20}D$ −22.0 in water, agreeing with Micheel's[4] data) by the hydrolytic conditions that were used in making D-mannosan from its acetone compound.[2] Periodate oxidation shows that the ring configurations for D-galactosan are $<1,5>\beta$-$<1,6>$. Acetone-D-galactosan $<1,5>\beta<1,6>$ possesses only one free hydroxyl group, the position of which is limited to one of three carbon atoms (2, 3 and 4).[5] The substance, now so readily available,[6] offers possibilities for syntheses, especially of disaccharides. The work is being continued.

(5) The recent research of McGreath and Smith, *J. Chem. Soc.*, 387 (1939), indicates that the free hydroxyl group is one carbon atom 2, the acetone having condensed on the *cis* hydroxyls of carbon atoms 3 and 4, as originally supposed by Micheel.

(6) Micheel's synthesis of D-galactosan starts from acetobromogalactose and trimethylamine.

NATIONAL INSTITUTE OF HEALTH
U. S. PUBLIC HEALTH SERVICE RAYMOND M. HANN
WASHINGTON, D. C. C. S. HUDSON

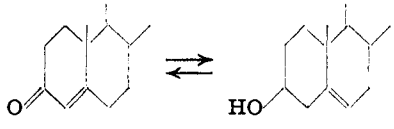
RECEIVED MARCH 25, 1941

STEROLS. CXXII

Sir:

Recently, Wolfe, Fieser and Friedgood [THIS JOURNAL, **63,** 583, 1941] raised a question as to our hypothesis on the formation of the Δ^5-3-hydroxysteroids as reduction products of a Δ^4-3-ketosteroid as lacking any foundation of analogy and as unlikely because "the process would require the migration of the double bond at 4,5, presumably after reduction of the carbonyl group, away from its position of conjugation with the oxygen atom."

We wish to point out that this type of reduction in the animal has been accomplished by Schoenheimer, Rittenberg and Graff [*J. Biol. Chem.*, **111,** 183 (1935)]. These authors fed coprostenone, a Δ^4-3-ketosteroid to a dog and found that it was eliminated as cholesterol, a Δ^5-3-hydroxysterol in which reduction of the ketone group had taken place with a migration of the double bond at 4,5.



They concluded that the formation of cholestenone from cholesterol is a biologically reversible process. Their work was discussed in our article on the theory of the formation of the various steroids [THIS JOURNAL, **60,** 1725 (1938)]. Although it is possible that the above reduction may be bacterial, it should be noted that it has never been proven that the various urinary steroidal reduction products are formed by glandular reduction and not by bacterial reduction.

In addition, it should be pointed out that according to our hypothesis of the formation of the various steroids, dehydroisoandrosterone need not be a transformation product of testosterone, or of androstenedione, but can arise directly as a degradation product of many of the numerous cortical steroids, by mechanisms described in our paper, without going through testosterone as an intermediate. The same is true for isoandrosterone, androsterone, etiocholanolones, etc. The numerous routes through which these products could be formed from the cortical compounds were omitted from our original paper, for the sake of brevity.

SCHOOL OF CHEMISTRY AND PHYSICS
THE PENNSYLVANIA STATE COLLEGE
STATE COLLEGE, PENNA. RUSSELL E. MARKER

RECEIVED FEBRUARY 21, 1941

ORIGIN OF DEHYDROISOANDROSTERONE IN URINE

Sir:

The observation of Schoenheimer, Rittenberg and Graff cited by Marker in the accompanying communication does not seem to us to constitute a valid reason for believing that the dehydroisoandrosterone secreted in urine arises from a Δ^4-3-ketosteroid precursor. The feeding of cholestone to a dog on a biscuit diet resulted in an increase in the fecal cholesterol over that noted in control periods; when the dog was put on a meat diet, cholestenone feeding increased the output of the principal fecal sterol, which in this case was coprosterol. The cholestenone used carried no indicator element, and no proof was adduced that the excess excretory sterols were transformation products of the material administered. Over 80% of the administered material remained unaccounted for, even on the supposition of a conversion, and the ketone may have stimulated normal sterol excretion, supplanted a normal transformation product of cholesterol, or influenced the sterol excretion in some other indirect manner. The experiment, therefore, cannot be considered to have established that cholestenone is capable of undergoing reduction to cholesterol

under the influence of intestinal bacteria. On the other hand, Mamoli and associates [*Ber.*, **71**, 156, 650, 2083, 2698 (1938)] have demonstrated, in a series of experiments in which the relationship of the starting material to the product of bio-transformation is not subject to question, that bacterial reduction of Δ^4-3-ketosteroids of the hormone series follows an entirely different course, in which the Δ^4-double bond invariably is saturated prior to reduction of the C_3-carbonyl group. The bio-reduction of androstenedione and of testosterone was studied in a number of instances, but in no case was dehydroisoandrosterone or other Δ^5-unsaturated steroid encountered as a reduction product.

Of still greater significance to the question of the origin of the dehydroisoandrosterone found in urine, is the direct experiment of N. H. Callow [*Biochem. J.*, **33**, 559 (1939)]. Callow found that administration of testosterone propionate to a male patient resulted in an unmistakable increase in the urinary excretion of androsterone and 3α-hydroxyaetiocholanone-17, but that there was no evidence of the conversion of the administered Δ^4-3-ketosteroid into dehydroisoandrosterone. Thus the present evidence, in our opinion, is contradictory to Marker's hypothesis.

CONVERSE MEMORIAL LABORATORY
HARVARD UNIVERSITY LOUIS F. FIESER
CAMBRIDGE, MASSACHUSETTS JOHN K. WOLFE

RECEIVED MARCH 26, 1941

A DETERMINATION OF THE HYDROXY AMINO ACIDS OF INSULIN

Sir:

We know of no previous attempts to identify or estimate the hydroxy amino acids of insulin. This is not surprising, since the material is not cheap, and suitable methods have been lacking.

On the basis of our observation[1] that periodic acid reacts, under suitable conditions, rapidly and quantitatively with hydroxy amino acids in the manner shown

$$\mathrm{RCH(OH)-CH(NH_2)-CO_2H} \xrightarrow{HIO_4} \mathrm{RCHO + NH_3 + OCHCO_2H}$$

it has been possible to develop suitable analytical methods. The ammonia evolved may be made to estimate the total of hydroxy amino acids (of the usual types) to be expected. And determinations of the individual aldehydes allow a somewhat accurate appraisal of serine and threonine.[2]

An application of these methods to insulin has given the results shown. All figures given are corrected for moisture and for ash.

TABLE I

"BALANCE SHEET" FOR HYDROXY AMINO ACIDS OF INSULIN[a]

Total hydroxy amino acids		7.75% SE[b,c]
Threonine	2.66%[d]	2.35% SE
Serine	3.57%[e]	3.57% SE
"Other" hydroxy amino acids (as serine)		1.83% SE

[a] Average values. [b] Calculated as "serine equivalent," SE. [c] Actual values, 7.88, 7.62%, SE. [d] Actual values, 2.69, 2.63, 2.68, 2.61, 2.67%. [e] Actual values, 3.52, 3.62%.

Du Vigneaud's excellent summary[3] of the known components of insulin as of 1938, with calculation of "residue numbers" of the amino acids in terms of the Bergmann–Niemann theory, showed 54 units in 288 not accounted for. Calculated in these terms, our results show: threonine, 8 units (found, 7.83); serine, 12 units (found, 11.94); *other* hydroxy amino acids, 6 units (found, 6.12).

It is only proper to add that, since the serine was really determined as formaldehyde, *any part* of the amount reported could be (in equimolecular proportion) hydroxylysine. We think it the simpler assumption that it is all serine.

We wish to express our thanks to Prof. V. du Vigneaud, who gave us the gram of crystalline insulin with which this work was done, and who supplied the data on moisture and ash for this sample which we have used in our corrections.

(1) Nicolet and Shinn, THIS JOURNAL, **61**, 1615 (1939).

(2) Shinn and Nicolet, *J. Biol. Chem.*, **138**, 91 (1941).

(3) Du Vigneaud, *Cold Spring Harbor Symposia on Quant. Biol.*, **VI**, 275 (1938).

BUREAU OF DAIRY INDUSTRY
U. S. DEPT. OF AGRICULTURE BEN H. NICOLET
WASHINGTON, D. C. LEO A. SHINN

RECEIVED APRIL 15, 1941

THE TRIPLE POINT PRESSURE OF HYDROGEN

Sir:

In view of the importance of the hydrogen triple point as a fixed point in thermometry, there is surprising lack of agreement on the value for the triple point pressure. The first five entries of Table I give some determinations of this constant.

The program of the State College Cryogenic Laboratory has involved frequent checks of the laboratory temperature scale against thermometric fixed points. The latter entries of Table I