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

give the value obtained for the hydrogen triple point pressure. The apparatus, method and accuracy have been described.[1] Since our manometric accuracy is about ±0.02 mm., the agreement shown is poor.

TABLE I

TRIPLE POINT PRESSURE OF NORMAL HYDROGEN

Pressure, int. mm.	Observed by
50.7	Kamerlingh Onnes and Keesom[2]
53.8	Simon and Lange[3]
54.86	Henning[4]
54.	Scott, *et al.*[5]
54.1	Henning and Otto[6]
	Cryogenic Laboratory, State College
53.83	(1) October 23, 1937
54.18	(2) October 23, 1937
54.23	(3) December 17, 1938
53.89	(4) August 15, 1940
53.93	(5) August 15, 1940
54.18	(6) August 15, 1940

The measurements of August, 1940, were made with the purpose of determining the cause of the uncertainty. The calorimeter contained 10 cc. of liquid hydrogen (previous measurements had been made with about this same quantity). After being cooled below the triple point, the cryostat was evacuated and the calorimeter allowed to drift slowly to the triple point temperature. Ten minutes after the temperature drift became zero, as determined by the constantan resistance thermometer on the calorimeter, the result (4) was obtained, in fair agreement with (1) which had previously been determined in the same way. Thirty minutes later point (5) was taken. Enough electrical energy was then supplied to the calorimeter to melt a third of the sample of hydrogen, after which point (6) was taken.

It appears that when an appreciable quantity of liquid was present, a vertical temperature gradient through the liquid layer on top of the solid in the calorimeter prevented the measurement of the true triple point pressure. This true pressure was obtained only when the quantity of liquid present was too small to allow the establishment of any appreciable thermal gradient. It seems likely that disagreement among the results of other workers is due to the same cause. We have accordingly taken 53.85 ± 0.03 int. mm. as the triple point pressure of normal hydrogen.

(1) Aston and Messerly, THIS JOURNAL, **58**, 2354 (1936); Messerly and Aston, *ibid.*, **62**, 886 (1940).
(2) *Comm. Phys. Lab. Univ. Leiden*, **137a** (1913).
(3) *Z. Physik*, **15**, 307 (1923).
(4) *Ibid.*, **40**, 775 (1927).
(5) *J. Chem. Phys.*, **2**, 454 (1934).
(6) *Physik. Z.*, **37**, 633 (1936).

In connection with the use of the triple point of hydrogen as a fixed point in thermometry, it is important to note that, depending upon the geometry of the apparatus, the triple point temperature may be measured whether or not the triple point pressure prevails; when the latter condition is fulfilled, however, the true triple point temperature is certainly measured.

(7) Present address: United States Bureau of Mines, Pittsburgh, Pa.

THE CRYOGENIC LABORATORY
SCHOOL OF CHEMISTRY AND PHYSICS
THE PENNSYLVANIA STATE COLLEGE
STATE COLLEGE, PENNSYLVANIA G. H. MESSERLY[7]

RECEIVED APRIL 5, 1941

PROCEDURE FOR THE PREPARATION OF CERTAIN DERIVATIVES OF STARCH

Sir:

Heretofore no solution or paste of starch in an inert organic solvent suitable for use in esterification or etherification reactions has been available. Attempts to gelatinize native starch directly in tertiary nitrogenous bases, such as pyridine, quinoline, etc., have not been successful, since even upon boiling the starch in such solvents the granules remain unburst. We have found that if first the starch granules are burst in various ways, such as by boiling in water, autoclaving, grinding, etc., it is possible to prepare solutions and pastes of starch in such solvents. A suitable procedure is to boil the native starch in water and then add pyridine and continue the boiling with distillation so as to eliminate the water as pyridine–water azeotrope boiling at 92–93°, thereby producing a solution or paste of starch in pyridine. The products so obtained range from clear solutions, when the pyridine contains a small amount of water (about 4%), to thick jellies, when the water is absent. Instead of treating the native starch, we may separate the alpha and beta amyloses thereof [Pacsu and Mullen, THIS JOURNAL, **63**, 1168 (1941)] and gelatinize each separately in the tertiary nitrogenous base. The starch gelatinized in tertiary bases is highly reactive toward esterification and etherification reagents, such as the anhydrides and acyl halides of aliphatic and aromatic acids, benzyl chloride, etc., and is found to be trifunctional giving rise to triesters and ethers in practically quantitative yield. The products so obtained are thermoplastic, yielding clear, glass-like substances, which appear to be of interest in the