marine eggs[13]) has a number of theoretical connotations in the chemistry of proteins and their biological manifestations.

(13) Tyler and Fox, *Biol. Bull.*, **79**, 153 (1940).

CHEMISTRY LABORATORY
IOWA STATE COLLEGE
AMES, IOWA
SIDNEY W. FOX
MARY JANE WARD

RECEIVED AUGUST 19, 1946

ELEMENTARY ISOTOPIC ANALYSIS. DETERMINATION OF OXYGEN

Sir:

At present *oxygen* in organic compounds is usually determined by difference, that is, after deducting the percentage of carbon, hydrogen and any other constituents from 100. The ter Meulen catalytic method,[1] proposed in recent years, is the only direct method, but it is complicated and requires careful manipulation.

In view of the availability of mass spectrographs and heavy oxygen, it occurred to us to develop an isotopic method. It is a very simple adaptation of the isotope dilution principle originally introduced by G. v. Hevesy and F. Paneth. A known weight (a) of the substance to be analyzed ($x\%$ O) is equilibrated with a known volume of oxygen gas ($= b$ g.), containing a known amount of O^{18} ($= m\%$) in *excess* of the normal concentration. The excess of O^{18} in the mixture after equilibration ($= n$) is determined by mass spectrograph. Thus a, b, m and n being determinable, it follows simply from the mixture rule that

$$x = \frac{b(m - n)100}{an} \% \text{ Oxygen} \qquad (1)$$

The equilibration takes place in a platinum test-tube of 80 ml. volume, at 600–800°, connected to a vacuum system. The time required is about one-half hour. The following oxygen determinations show results obtainable by this method:

Detn. no. Substance analyzed	1 Formic acid HCOOH (excess of O_2)	2 Formic acid HCOOH (deficiency of O_2)	3 Acetic acid CH_3COOH	4 1-Nitroethane $CH_3CH_2NO_2$	5 Ethyl ether $(C_2H_5)_2O$
a, *i. e.*, mg. sample	38.2	60.4	26.0	20.9	52.70
b, mg. oxygen-18 gas taken (using at. wt. of O = 16.00)	20.0	22.2	36.5	31.4	12.03
(ml. at N. T. P.)	(14.0)	(15.5)	(25.5)	(22.0)	(9.4)
m, mole % $O^{18}\cdot O^{16}$ in b above normal concn. (-0.40%)	2.00	2.00	2.07	2.10	2.14₅
n, mole % $O^{18}\cdot O^{16}$ of equilibrated mixt. (-0.40%)	0.85	0.69	1.50	1.64	1.15
x, % oxygen in substance, calcd. from formula (1)	70.8	69.8	53.4	40.3	19.8
% oxygen, theoretical.	69.5	69.5	53.3	42.6	21.6

These experiments, particularly 2 and 3, show even better agreement than is to be expected. With the comparatively small O^{18} concentration available to us, the average probable error is ±3.0% of the oxygen content.

Since ($m - n$) and n enter into our equation, the accuracy is highest when the amount of isotopically labeled element added approximately equals the element content of the sample. The precision of oxygen determinations can be increased simply by production of O^{18} in high concentration. With about 20 at. per cent. O^{18} an average precision of ±0.1% of the oxygen content of the sample analyzed should be attainable.

The two experiments with formic acid show that it is not necessary to completely combust the sample to CO_2 and H_2O. As in Experiment 1, equilibrium is attained, with CO and CH_4 in the gas mixture.

The most reliable O^{18} figures are obtained from the CO_2-peaks. The same method may be used for solid organic compounds, and of course can be extended to inorganic compounds which would equilibrate with an O^{18}-containing molecule.

Description of our equipment and our detailed results will be published elsewhere. The method is now being extended to the determination of carbon, hydrogen and nitrogen.

We are greatly indebted to Dr. Harry Thode of Toronto, Canada, for a generous supply of H_2O^{18}, without which this investigation would not have been possible.

THE HOUDRY LABORATORIES
LINWOOD, PA.
A. V. GROSSE
S. G. HINDIN
A. D. KIRSHENBAUM

RECEIVED AUGUST 30, 1946

BUBBLE FORMATION FROM CONTACT OF SURFACES

Sir:

If commercial bottled soda water is poured carefully into a specially *cleaned wet* glass container the liquid remains free of bubbles and supersaturated with carbon dioxide, in contrast with soda water in an ordinary tumbler, where gas phases (gas nuclei) are present to start bubble formation. A clean wet quartz rod in the soda water will also form no bubbles. However, if the quartz rod is drawn over the surface of the glass so as to produce a scratch, bubbles arise at the contact of the two surfaces and a chain of bubbles may continue to form at points along the scratch for a considerable time period.

The above observations are widely known. We have, however, now been able to show that bubbles

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(1) For a comprehensive and critical review of this and other less developed methods see: P. J. Elving and W. B. Ligett, *Chem. Rev.*, **34**, 129–156 (1944).

do not come merely from a newly cut surface. If the same type of scratch is made on clean glass covered with a thin layer of pure water, and a large volume of soda water immediately poured carefully over the surface, no bubbles arise from the scratch. Bubbles form only during the process of chipping the glass.

Since intense sound waves passing through water cause cavitation and bubbling, we might suppose the phenomenon to be the result of sound waves generated by the scratch. It is difficult to imagine that such sound waves can be intense enough. Moreover, a scratch on the outside of a thin glass container of soda water will not cause bubbles to form inside, provided the scratch does not actually cut through the glass so that an air phase comes in contact with the liquid.

Bubble formation from contact of surfaces becomes more marked in water supersaturated with very high pressures of gas or in water containing air at one atmosphere, evacuated to its vapor pressure. The mere tap of a glass rod on a glass wall will induce bubbles under these conditions. In one experiment, a small glass dish of water covered with cellophane and containing a sphere of plexiglas (5 mm. diameter) was saturated with 80 atmospheres of nitrogen gas and then decompressed to 1 atmosphere. No bubbles formed because the glass dish, water, cellophane and plexiglas sphere had been well cleaned and previously subjected to 16,000 lb./sq. in. hydrostatic pressure (to force gas nuclei into solution).[1] However, when the dish was tipped slightly so that the plexiglas sphere rolled over the glass surface, bubbles arose from its path and a burst of bubbles appeared when it hit the side of the dish.

It is inconceivable that chips of glass can be broken off under a light rolling sphere of plexiglas. It is more likely that when surfaces touch and then separate, the surface layer of water is pushed aside forming a momentary dry spot. Before the water can move in again, a sufficient number of gas molecules accumulate in the space, thereby forming a gas nucleus which immediately grows into a bubble.

[1] Harvey, *et al.*, THIS JOURNAL, **67**, 156 (1945).

PHYSIOLOGICAL LABORATORY E. NEWTON HARVEY
PRINCETON UNIVERSITY KENNETH W. COOPER
PRINCETON, NEW JERSEY ARTHUR H. WHITELEY

RECEIVED SEPTEMBER 14, 1946

DEGRADATIVE STUDIES ON STREPTOMYCIN

Sir:

Folkers and co-workers[1] have advanced the formula $(C_{21}H_{37-39}O_{12}N_7 \cdot 3HCl)_2 \cdot CaCl_2$ for the calcium chloride double salt of streptomycin trihydrochloride (I). Our analytical data on purified samples of I, $[\alpha]^{25}D$ −81° (*c* 2, water), support the above formula and establish the presence of one methyl group linked to carbon.

Anal. Calcd. for $(C_{21}H_{37}O_{12}N_7 \cdot 3HCl)_2 \cdot CaCl_2$: C, 33.88; H, 5.42; N, 13.17; Cl, 19.05; Ca, 2.69. Calcd. for $(C_{21}H_{39}O_{12}N_7 \cdot 3HCl)_2 \cdot CaCl_2$: C, 33.79; H, 5.67; N, 13.14; Cl, 19.00; Ca, 2.68. Found: C, 33.82; H, 5.69; N, 12.94; Cl, 18.64; Ca, 2.65. A C-methyl determination gave 1.9% CH_3 (calcd. for one C-methyl group, 2.0%).

Hydrogenation of an aqueous solution of I at 100–140 atmospheres and 150° with Raney nickel catalyst, followed by methanolysis and acetylation with acetic anhydride and pyridine yields crystalline methyl dihydrostreptobiosaminide pentaacetate, m. p. 194–195°, $[\alpha]^{28}D$ −117° (*c* 2, chloroform).

Anal. Calcd. for $C_{13}H_{19}O_8N(CH_3CO)_5OCH_3$: C, 51.15; H, 6.61; N, 2.49; OCH_3, 5.51; O-acetyl, 30.5. Found: C, 51.04; H, 6.93; N, 2.50; OCH_3, 5.86; O-acetyl, 30.6.

This compound is unchanged after treatment with hot acetic anhydride and sodium acetate. Since methanolysis and acetylation of streptomycin[2] is known to yield a tetraaacetate containing three methoxyl groups, it appears that in the above compound a carbonyl group of streptomycin has been reduced to an alcohol group.

Mercaptolysis of I with subsequent acetylation[3] yields, after chromatographic purification,[4] two forms of ethyl thiostreptobiosaminide diethyl thioacetal tetraacetate: A, m. p. 80.5–81°, $[\alpha]^{31}D$ −192° (*c* 2, chloroform); B, m. p. 111–111.5°, $[\alpha]^{28}D$ −29° (*c* 3, chloroform).

Anal. Calcd. for $C_{13}H_{18}NO_7(C_2H_5S)_3(CH_3CO)_4$: C, 49.44; H, 6.92; N, 2.14; S, 14.66; CH_3CO, 26.3; mol. wt., 655.9. Found for A: C, 49.63; H, 6.83; N, 2.21. Found for B: C, 49.54; H, 6.71; N, 2.15; S, 14.75; CH_3CO, 26.2; mol. wt. (Rast), 651.

These data indicate that in streptomycin, streptidine is attached to a glycosidic hydroxyl (cyclic hemiacetal) capable of existing in anomeric forms. Formation of a thioglycoside rather than a thioacetal indicates that the cyclic structure concerned in this hemiacetal linkage resists hydrolysis.

Hydrogenolysis of either A or B above with Raney nickel catalyst[5] followed by reacetylation yields didesoxydihydrostreptobiosamine tetraacetate (II), m. p. 158.5–159°, $[\alpha]^{31}D$ −86° (*c* 3, chloroform).

Anal. Calcd. for $C_{13}H_{21}O_7N(CH_3CO)_4$: C, 53.04; H, 7.00; N, 2.94; O-acetyl, 27.2. Found: C, 53.06; H, 7.02; N, 3.04; O-acetyl, 25.4.

An attempted high-pressure hydrogenation of II at 150° with Raney nickel gives unchanged material, demonstrating the absence of an olefinic linkage in the molecule.

[1] R. L. Peck, N. G. Brink, F. A. Kuehl, Jr., E. H. Flynn, A. Walti and K. Folkers, THIS JOURNAL, **67**, 1866 (1945).

[2] N. G. Brink, F. A. Kuehl, Jr., and K. Folkers, *Science*, **102**, 506 (1945).

[3] M. L. Wolfrom and J. V. Karabinos, THIS JOURNAL, **67**, 500 (1945).

[4] W. H. McNeely, W. W. Binkley and M. L. Wolfrom, *ibid.*, **67**, 527 (1945).

[5] M. L. Wolfrom and J. V. Karabinos, *ibid.*, **66**, 909 (1944).