The micrographs indicate that fibrin is produced through a predominantly lateral association of fibrinogen filaments. The characteristic striation does not appear under all circumstances, for example, in fibrils with widths below about 200 Å., or in tapered ends with widths less than this. Unstriated fibrils show a randomly particulate structure, while in the striated portions the particles are more concentrated in the stained bands. The micrographs of fibrinogen do not show any degree of regularity either in length or internal structure comparable to the regularity in fibrin. It appears that the periodicity in fibrin is not a manifestation of rigid dimensional units in fibrinogen, but is, rather, a characteristic developed subsequent to initial aggregation The anomalous variations in protein concentration indicate that some of the constituents have experienced local axial shifts to preferred positions.

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RECEIVED FEBRUARY 12, 1949

FERMENTATION OF GLUCOSE-1-C^{14}

Sir:

We have synthesized *d*-glucose-1-C^{14} from *d*(−)-arabinose by the Fischer–Kiliani method.[1] The mixed glucono- and mannonolactones were reduced catalytically,[2] carrier glucose added, and the radioglucose isolated and recrystallized to constant specific activity (about 6000 counts per gram minute). The glucose (in 0.2-g. samples) was then fermented anaerobically by Fleischmann's baker's yeast in phosphate buffers in the absence of a source of combined nitrogen; the fermentation yields (based on the carbon dioxide obtained) were 75–90%. The Embden–Meyerhof fermentation mechanism[3] predicts that all the radiocarbon will appear in the methyl group of the alcohol so obtained; this prediction has been substantially confirmed.

The carbon dioxide obtained directly in the fermentation was counted as $BaCO_3$. The alcohol was degraded to acetone by the method outlined below; the latter was counted as its 2,4-dinitrophenylhydrazone.

$$C^{14}H_3CH_2OH \xrightarrow[H_2SO_4]{K_2Cr_2O_7} C^{14}H_3CO_2H \xrightarrow{Ba(OH)_2} (C^{14}H_3CO_2)Ba(O_2CCH_3) \xrightarrow[\text{Vacuum}]{530^\circ} C^{14}H_3COCH_3 + BaCO_3$$

CH_3CH_2OH CH_3CO_2H

In a set of control experiments on the pyrolysis using methyl-labelled acetic acid,[4] we found 0.1–0.3% of the activity in the barium carbonate produced. Further in these control experiments, the specific activity of the acetone 2,4-dinitrophenylhydrazone was only 77% of that anticipated from the specific activity of the barium acetate, even after making the usual corrections[5] for self-absorption, etc. The specific activities of all samples of acetone 2,4-dinitrophenylhydrazone were therefore corrected by the factor 1/0.77. The final results, together with the counting errors (95% confidence level), are given below:

*p*H	Other conditions	Per cent. of radioactivity (based on glucose fermented) found in CO_2	CH_3	CH_2OH
6.2	Live yeast	6.5 ± 2.4	92 ± 4.0	4.8 ± 2.4
5.7	Live yeast	1.0 ± 1.8	76 ± 4.0	7.6 ± 2.8
5.7	Dried yeast powder	3.7 ± 1.8	92 ± 7.8	0.2 ± 0.4

We are currently investigating the causes of the slight radioactivity in the carbon dioxide obtained directly in the fermentation and in the barium carbonate from the pyrolysis.

(1) Kiliani *Ber.*, **19**, 3033 (1886).
(2) Glattfeld and Schimpf, THIS JOURNAL, **57**, 2204 (1935).
(3) Meyerhof, *Biochem. Symposia*, **V**, 141 (1941).
(4) The methyl labelled acetic acid was kindly supplied to us by Professor Konrad Bloch
(5) Yankwich and Weigl, *Science*, **107**, 651 (1948); Libby, *Ind. Eng. Chem., Anal. Ed.*, **19**, 2 (1947).

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A NEW SYNTHESIS OF 2-PHENAZINOL, THROUGH THE DI-N-OXIDE

Sir:

The chlorine atom of 2-chlorophenazine can be labilized toward aqueous-alcoholic sodium or potassium hydroxide by converting the base to 2-chlorophenazine-5,10-dioxide (red-orange needles, m. p. 190–191° (dec.)[1] *Anal.*[2] Calcd. for $C_{12}H_7ClN_2O_2$: C, 58.4; H, 2.87. Found: C, 58.5; H, 3.06. This conversion is readily effected, using the method employed by Clemo and McIlwain[3] for other phenazines. Refluxing the chlorophenazine dioxide for about twelve hours with aqueous-alcoholic potassium or sodium hydroxide gives a deep purple solution of the alkali salt, from which hydrochloric acid precipitates the free 2-phenazinol-5,10-dioxide (orange-red, begins to darken at 236°). *Anal.* Calcd. for $C_{12}H_8N_2O_3$: C, 63.2; H, 3.54. Found: C, 63.1; H, 3.87. Reduction of this by sodium hyposulfite (sodium "hydrosulfite") in alkaline solution at room temperature gives a red solution of the sodium salt of 2-phenazinol, from which the free phenol[4] is precipitated by acid. (Clemo and McIlwain[3] found sodium hyposulfite effective in reducing 1-phenazinol-5,10-dioxide.) The 2-phenazinol may be purified by vacuum sublimation and chromatographic adsorp-

(1) All melting points are corrected.
(2) Analyses by Mr. W. C. Alford, Mrs. M. M. Ledyard and Mrs. E. G. Peake.
(3) Clemo and McIlwain, *J. Chem. Soc.*, **483** (1938).
(4) Kehrmann and Cherpillod, *Helv. Chim. Acta*, **7**, 975 (1924). As these authors indicate, in saying that the 2-phenazinol melts at "about" 253-254° with decomposition, this compound does not appear to have a sharp melting point.

tion on alumina. *Anal.* Calcd. for $C_{12}H_8N_2O$: C, 73.4; H, 4.11. Found: C, 73.8; H, 4.25.

The 2-chlorophenazine was made by the method of Waterman and Vivian,[5] starting from 4-chloro-2-nitrodiphenylamine.

The antitubercular activity of the phenazine dioxides[6] makes both of the new dioxides reported above of interest, especially the alkali-soluble hydroxy compound; and by this synthesis the 2-phenazinol itself is rendered more easily accessible.

(5) Waterman and Vivian, *J. Org. Chem.*, March, 1949; U. S. Patent 2,292,808 (Aug. 11, 1942).

(6) Ihland, *Nature*, **161**, 1010 (June 26, 1948). Antitubercular activity of certain other phenazines: Barry, Belton, Conalty and Twomey, *ibid.*, **162**, 622 (Oct. 16, 1948).

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PREPARATION OF HIGH-PURITY HYDROGEN DEUTERIDE FROM LITHIUM ALUMINUM HYDRIDE

Sir:

Hydrogen deuteride of very high purity has been prepared previously by the fractional distillation of a mixture of H_2, D_2, and HD at liquid hydrogen temperatures.[1] We have now found that very pure HD can be prepared more simply by the action of certain metallic hydrides on heavy water. Thus, sodium hydride with heavy water gives 87% HD with H_2 and D_2 as impurities. Lithium aluminum hydride with heavy water gives 93% HD in some cases and in others 97%, with 2.5% H_2 + 0.5% D_2.[2] Determinations were made on a consolidated mass spectrometer for which instrumental condition were: magnet current of 0.15 ampere and voltages of 3800 for mass 1 (H^+), 1900 for mass 2 (H_2^+ and D^+), 1267 for mass 3 (HD^+), and 950 for mass 4 (D_2^+).

It was suspected that the heat evolved in the reaction promoted the formation of D_2 and H_2. Accordingly the preparation with lithium aluminum hydride was carried out in a bath at 0°, with the resulting formation of 99% pure HD. The exact nature of the temperature dependence is not known, but the variation in composition of the gas product may be due to a shifting of the $H_2 + D_2 \rightleftarrows HD$ equilibrium, as well as to the presence of impurities in the lithium aluminum hydride.

Our preferred procedure for this preparation, therefore, was as follows:

Pure D_2O (99.8%) contained in a hypodermic syringe was injected through a neoprene serum stopper, into a stirred ice-cold slurry of lithium aluminum hydride in *n*-butyl ether contained in the reaction flask of a modified Zerewitinoff apparatus.[3] After the vigorous reaction subsided, the evolved gas was collected in an evacuated sample bottle.

This very simple preparation of HD from lithium aluminum hydride and knowledge of its fragmentation pattern should improve the mass spectrometric analyses of H_2, D_2 and HD mixtures, especially those low in H_2.

We wish to acknowledge the helpful assistance of A. G. Sharkey, Jr.

(1) Scott and Brickwedde, *Phys. Rev.*, **48**, 483 (1935).

(2) Beutler, Brauer and Junger, *Naturwissenschaften*, **24**, 347 (1936) reported the preparation of a mixture rich in HD by the action of lithium hydride on heavy water.

(3) Orchin and Wender, *Anal. Chem.*, in press.

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