retical amount of water was collected. The reaction mixture was then cooled, washed with dilute bicarbonate solution and dried over anhydrous sodium sulfate. The product was isolated by vacuum distillation. Yields were in the neighborhood of 80–90%.

TABLE I

| Esters of | B. p. °C. | B. p. mm. | Chlorine, % Calcd. | Chlorine, % Found |
|---|---|---|---|---|
| Monochloroacetic acid | | | | |
| 2-Phenylcyclohexyl | 141–143 | 1.5 | 14.05 | 14.38 |
| Butyl cellosolve | 85–87 | 1.5 | 18.25 | 18.10 |
| Dichloroacetic acid | | | | |
| 2-Phenylcyclohexyl | 149–151 | 1.5 | 24.70 | 24.42 |
| Cyclohexyl | 80–82 | 1.5 | 33.60 | 33.76 |
| Butyl cellosolve | 90–93 | 1.5 | 31.00 | 31.25 |
| Trichloroacetic acid | | | | |
| 2-Phenylcyclohexyl | 157–159 | 1.5 | 32.50 | 32.70 |
| Cyclohexyl | 85–88 | 2 | 43.00 | 43.30 |

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RECEIVED NOVEMBER 5, 1948

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### 1-Benzyl-3-phenyl-2-thiohydantoin

**Ethyl N-Benzylglycinate.**—Ethyl chloroacetate was condensed with benzylamine according to the directions of Mason and Winder.[1] The yield of ethyl N-benzylglycinate on the second distillation, 128–131° at 6 mm., was 55%. The picrate derivative was prepared by mixing ethereal solutions of the two substances and scratching to induce crystallization. These yellow plates, washed with ether, melted 166–168° (micro-block).[2] A benzoyl derivative was also attempted but it could not be prepared by either the Schotten–Baumann method or by boiling with benzoyl chloride in benzene solution. This unexpected behavior prompted the attempt to form a thiourea derivative.

**1-Benzyl-3-phenyl-2-thiohydantoin.**—Approximately equal amounts of ethyl N-benzylglycinate and phenyl isothiocyanate were mixed in alcoholic solution and boiled for a minute or two. The product which separated on cooling was recrystallized from alcohol to give a good yield of long flat needles, m. p. 188.5–189.5° (micro-block). Instead of the expected thiourea the product indicated by analysis was the 2-thiohydantoin which resulted from cyclization of the thiourea by splitting out of ethanol. This type of cyclization is not unusual, though it generally requires higher temperatures[3] or acid catalysis.[4] *Anal.* Calcd. for $C_{16}H_{14}N_2OS$: C, 68.06; H, 5.00; N, 9.92. Found: C, 68.05; H, 5.12; N, 9.93.

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RECEIVED NOVEMBER 9, 1948

(1) Mason and Winder, *J. Chem. Soc.*, 188 (1894).

(2) Slight decomposition—as evidenced by the evolution of a distillate—was noticeable as low as 150°. This might bear some relationship to the 154° m. p. reported by Mason and Winder.[1]

(3) Wheeler and Brautlecht, *Am. Chem. J.*, **45**, 446 (1911).

(4) Morton, "The Chemistry of Heterocyclic Compounds," McGraw-Hill Book Co., N. Y., 1946, p. 459.

(5) Present address: Department of Chemistry, University of Wisconsin, Madison, Wis.

# COMMUNICATIONS TO THE EDITOR

## STUDIES OF FIBRINOGEN AND FIBRIN WITH THE ELECTRON MICROSCOPE

*Sir:*

Electron micrographs of metal-shadowed bovine Fraction I and human fibrinogen have been obtained showing that these materials consist largely of filamentous elements which are nodous in outline, somewhat like a string of beads, about 40 Å in diameter. Since the filaments tend to intermingle, it is difficult to measure all lengths with certainty, but such filaments as can be discerned vary in length from 300 to 1100 Å., with an average of about 600 Å. From flow-birefringence data it has been concluded that the fibrinogen particle can be represented by a prolate ellipsoid with a major axis of 700 Å. and an axial ratio of 18:1.[1] The widths observed are not appreciably different from the calculated minor axis. Numerous filaments occur with lengths close to the 700 Å. predicted, but the correlation is unsatisfactory in that flow-birefringence data indicate a constancy in length, while the electron microscope observations show a distribution of lengths. The discrepancy might be due to differences in samples or to difficulties inherent in the electron microscope methods.

An axial periodicity of about 250 Å. has been reported in bovine fibrin[2] after staining with phosphotungstic acid. The macroperiod is superficially similar to that in collagen[3] and certain other protein fibrils. In the present investigation, electron micrographs were obtained with improved resolution, showing that in bovine and human fibrin, the macroperiod consists of narrow stain-receptive bands midway between denser and wider stain-receptive bands whose average distance center-to-center along the fibril axis is about 230 Å. The spacing is constant to about 3% in individual fibrils, but varies by as much as 20% between separate fibrils. The fibrils appear to consist of particles having diameters in the range 30 to 50 Å. Metal-shadowing shows that the stain-receptive bands represent higher portions, indicating that the intervening regions have shrunk during drying. It is concluded that the periodic structure represents regular fluctuations in protein concentration in the originally hydrated system.

(1) J. T. Edsall, J. F. Foster and H. Scheinberg, THIS JOURNAL, **69**, 2731 (1947).

(2) C. V. Z. Hawn and K. R. Porter, *J. Exp. Med.*, **86**, 285 (1947).

(3) C. E. Hall, M. A. Jakus and F. O. Schmitt, THIS JOURNAL, **64**, 1234 (1942).

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.

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

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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RECEIVED FEBRUARY 3, 1949

## 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.