The structure of the α-carbethoxyindole IV was established by saponification to the acid (m.p. 262°. *Anal.* Found: C, 70.8; H, 4.9; N, 7.3; equiv. wt., 188) and decarboxylation to 1,2-dihydropyrrolo[3,2,1-h,i]indole (VII) (m.p. 76–77°. *Anal.* Found: C, 83.8; H, 6.1; N, 9.6). In each case, the ultraviolet absorption was typical for a substituted indole and practically identical with that of the corresponding compound of the six-membered ring series (VIII).[9]

(9) G. Barger and E. Dyer, THIS JOURNAL, **60**, 2414 (1938).

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A DIFFERENCE IN BOVINE AND HUMAN FIBRINOPEPTIDES WITH RESPECT TO THE OCCURRENCE OF TYROSINE-O-SULFATE

Sir:

Bettelheim[1] has reported tyrosine-O-sulfate to be a component of fibrinopeptide B, one of the peptides liberated by the action of thrombin on bovine fibrinogen. Recently Blombäck and Vestermark[2] also have reported evidence for the presence of tyrosine-O-sulfate in bovine fibrinopeptide B.

We have confirmed the presence of a component with the properties of tyrosine-O-sulfate in fibrinopeptide of bovine origin but have been unable to detect this compound in peptide derived from human fibrinogen.

Our initial experiments with bovine fibrinopeptide appeared to indicate the presence of a component, readily hydrolyzed by acid, but yielding a product other than tyrosine. The source of these difficulties proved to be the large amount of carbohydrate impurities in clinical thrombin (bovine origin, Parke, Davis and Co.). This material on acid hydrolysis yielded ultraviolet absorption maxima at 283 and 286 mμ in acid and alkaline solution, respectively, which is believed to be due to formation of hydroxymethylfurfural. These reactions either completely obscured or destroyed the liberated tyrosine. Purification of the thrombin according to Rasmussen[3] on Amberlite XE-64 (Rohm and Haas Co.) resulted in removal of interfering materials and a high purity thrombin.[4] Bovine fibrinogen was purified by the procedure of Laki[5] and was 94–96 per cent. clottable. Fibrinopeptide was isolated by the procedure of Bettelheim[6] using purified thrombin. The resulting fibrinopeptide mixture possessed the properties described by Bettelheim[1] with respect to ultraviolet spectra before and after acid hydrolysis. Chromatography of a barium hydroxide hydrolysate of fibrinopeptide on Dowex 1 acetate yielded a peak corresponding in position to that of synthetic tyrosine-O-sulfate.

(1) F. R. Bettelheim, THIS JOURNAL, **76**, 2838 (1954).
(2) B. Blombäck and A. Vestermark, *Arkiv for Kemi*, **12**, 173 (1958).
(3) P. S. Rasmussen, *Biochim. Biophys. Acta*, **16**, 157 (1955).
(4) W. H. Seegers and W. G. Levine, *Seventh Ann. Symposium on Blood*, Wayne State University, Detroit, 1958.
(5) K. Laki, *Arch. Biochem.*, **32**, 317 (1951).
(6) F. R. Bettelheim, *Biochim. Biophys. Acta*, **19**, 121 (1956).

Electrophoresis at *p*H 4.1, using the procedure of Bettelheim,[6] yields two bands with peptide of both bovine and human origin. The mixture of fibrinopeptides isolated from human fibrinogen purified with ammonium sulfate according to Laki[5] or with alcohol according to Morrison, *et al.*,[7] has shown no spectral evidence for tyrosine either before or after mild acid hydrolysis.

Other investigators[8–11] have shown that bovine fibrinogen possesses N-terminal glutamic acid and tyrosine while human fibrinogen possesses N-terminal alanine and tyrosine. The observations reported here suggest that other differences in chemical composition exist in fibrinogen of bovine and human origin.

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(7) P. R. Morrison, J. T. Edsall and S. G. Miller, THIS JOURNAL, **70**, 3103 (1948).
(8) K. Bailey, F. R. Bettelheim, L. Lorand and W. R. Middlebrook, *Nature*, **167**, 233 (1951).
(9) L. Lorand and W. R. Middlebrook, *Biochem. J.*, **52**, 196 (1952).
(10) L. Lorand and W. R. Middlebrook, *Science*, **118**, 515 (1953).
(11) B. Blombäck, *Arkiv for Kemi*, **12**, 299 (1958).
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A NEW PROCEDURE FOR FORMING CARBON-CARBON BONDS[1]

Sir:

In connection with our studies in oxindole chemistry[2] we have uncovered a novel method of monoalkylation of active methylene compounds. An investigation of a new synthesis of oxindole (Ia) by a desulfurization of isatin ethylenethioketal (II), m.p. 200–201° (Found: C, 53.92; H, 4.17; N, 6.50) revealed that a Raney nickel treatment of II in benzene or for four hours in ethanol gave the desired product. However, longer runs with W-2 Raney nickel and II, or even Ia, in a 10:1 weight ratio, in various alcoholic solutions yielded 3-alkyloxindoles (I).

Table I illustrates that (a) oxindole is an intermediate in the conversion of II to Ib-d; (b) a primary alcohol reacts faster than a secondary carbi-

(1) This work was supported by a research grant from the National Institutes of Health, Public Health Service, Department of Health, Education and Welfare (M1301).
(2) *Cf.* E. Wenkert, B. S. Bernstein and J. H. Udelhofen, THIS JOURNAL, **80**, 4899 (1958).