activity.[2] However, it is worth noting that much higher concentrations of thrombin are required to hydrolyze LEe than are needed to hydrolyze TAMe[3] or to activate fibrinogen[8] at the same *p*H. It is interesting to speculate that, while the primary action of thrombin on fibrinogen may be toward arginyl bonds, a slower secondary action may take place at lysyl bonds. If so, the latter activity may possibly be the origin of the phenomenon reported by Guest and Ware[5] wherein purified thrombin at very high concentration caused the lysis of fibrin clots, an action which was not prevented by STI.

In support of this additional specificity for thrombin we may cite some preliminary experiments involving Seegers' citrate thrombin and the oxidized B chain of insulin, prepared as described elsewhere.[9] Thrombin (50–100 TAMe units ml.) was incubated with oxidized B-chain (4–8 mg./ml.) at *p*H 8 in 0.1 *M* ammonium acetate for 18–24 hr. at 25°. In some experiments STI was added (0.8 mg./ml.) Parallel experiments were carried out using trypsin (0.1–0.2 mg./ml) in place of thrombin. The hydrolysis of the lysyl-alanine bond of the B-chain was assessed by detection of the alanine fragment by paper chromatography of the free amino acid and also of its DNP-derivative. With either method the intensity of the spot was undiminished by the presence of STI when thrombin was used rather than trypsin. No quantitative data are yet available for the degree of hydrolysis of the lysyl-alanine bond. Qualitatively, it appeared that thrombin was 10–20% as effective as trypsin under the conditions stated.

The activity of thrombin toward the arginyl-glycine bond of the B-chain is still under investigation. Further studies are also being carried out on the activity of thrombin toward synthetic lysyl substrates. We are indebted to Dr. W. H. Seegers for his generous gifts of purified thrombin.

(8) E. Mihalyi, quoted by J. A. Gladner and K. Laki, *Arch. Biochem. and Biophys.*, **62**, 501 (1956).

(9) S. J. Leach and H. A. Scheraga, *Compt. rend. Lab. Carlsberg*, in press.

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THE AZEOTROPE OF MONOCHLORODIFLUOROMETHANE AND DICHLORODIFLUOROMETHANE

Sir:

There is an azeotrope of monochlorodifluoromethane and dichlorodifluoromethane. That these two common refrigerants form an azeotrope has not been generally recognized, since the feasibility of their separation by simple distillation has been tacitly assumed in both the technical and patent literature in various instances. Furthermore, the azeotrope may occur in practical refrigeration systems, since dichlorodifluoromethane is sometimes added to monochlorodifluoromethane, when the latter is used as a refrigerant, in order to improve the low-temperature solubility of lubricating oil.

The existence of the azeotrope was demonstrated in two ways.

Reflux boiling points were measured, Table I, showing a minimum at about −41.4°, only 0.6° below the boiling point of monochlorodifluoromethane, at a composition of about 25% dichlorodifluoromethane by weight. There is little change in boiling point between 10 and 50% dichlorodifluoromethane by weight, the values lying between about −41.0 and −41.4°.

The existence of the azeotrope was confirmed by fractionating a mixture of 58.2% dichlorodifluoromethane and 41.8% monochlorodifluoromethane at high reflux in a low-temperature Podbielniak still (Cat. No. 407). Portions of the constant-boiling distillate analyzed 24.5 to 26.7% dichlorodifluoromethane by weight on the basis of the density of the gas and 25 to 29% dichlorodifluoromethane by weight based on infrared absorption. These results are in accord with expectation from the boiling-point data.

TABLE I

NORMAL BOILING POINTS OF MIXTURES OF MONOCHLORODIFLUOROMETHANE AND DICHLORODIFLUOROMETHANE

Weight % dichlorodifluoromethane in mixture	Boiling point °C.	Weight % dichlorodifluoromethane in mixture	Boiling Point °C.
0.0	−40.80	51.6	−40.73
1.4	−40.76	53.9	−40.63
2.9	−40.80	57.5	−40.54
5.2	−40.94	58.4	−40.54
6.9	−40.89	64.2	−39.84
9.0	−40.99	69.9	−38.93
15.0	−41.31	73.1	−38.33
21.9	−41.41	78.4	−37.14
27.8	−41.41	83.9	−35.87
32.5	−41.39	89.4	−34.26
38.8	−41.39	100.0	−29.80
44.4	−40.93		

"FREON" PRODUCTS LABORATORY
E. I. DU PONT DE NEMOURS & CO., INC.
WILMINGTON, DELAWARE B. J. EISEMAN, JR.

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STUDIES ON POLYPEPTIDES. XI. PREPARATION OF AN OCTAPEPTIDE POSSESSING MELANOCYTE-STIMULATING ACTIVITY[1]

Sir:

Structural studies of the corticotropins[2–5] and of the melanocyte-stimulating hormones (α- and β-M.S.H.)[6–8] have shown that the molecules of these substances contain a common amino acid sequence ("core") possessing the structure met-glu-his-phe-arg-try-gly. Since all these hormones stimulate

(1) Supported by grants from the U. S. Public Health Service, the National Science Foundation, Armour and Company, and Eli Lilly and Company.

(2) P. H. Bell, THIS JOURNAL, **76**, 5565 (1954).

(3) W. F. White and W. A. Landmann, *ibid.*, **77**, 1711 (1955).

(4) R. G. Shepherd, S. D. Willson, K. S. Howard, P. H. Bell, D. S. Davies, S. B. Davis, E. A. Eigner and N. E. Shakespeare, *ibid.*, **78**, 5067 (1956).

(5) C. H. Li, I. I. Geschwind, R. D. Cole, I. D. Raacke, J. I. Harris and J. S. Dixon, *Nature*, **176**, 687 (1955).

(6) J. I. Harris and A. B. Lerner, *ibid.*, **179**, 1346 (1957).

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(8) I. I. Geschwind, C. H. Li and L. Barnafi, THIS JOURNAL, **78**, 4494 (1956).