

tion mixture was filtered hot, concentrated and cooled. The precipitated solid was collected by filtration; 15.0 g. (87% yield) was obtained, which decomposed unsharply at about 200°.

Anal. Calcd. for $C_{11}H_{16}N_2O \cdot HCl$: C, 57.8; H, 7.5. Found: C, 58.1; H, 7.4.

***p*-Amino- β -diethylaminopropiophenone Hydrochloride.**—Reduction of β -diethylamino-*p*-nitropropiophenone hydrochloride exactly as described above was rapid and exothermic, being complete in 15 minutes. The product was isolated by diluting the filtered reaction mixture with ether. Recrystallization of the precipitated solid from isopropyl alcohol gave material melting at 134.5–136.0° dec.

Anal. Calcd. for $C_{13}H_{20}N_2O \cdot HCl$: C, 60.8; H, 8.3. Found: C, 60.5; H, 8.5.

Hydrogenation over Platinum Oxide.—Both β -dimethylamino- and β -diethylamino-*p*-nitropropiophenone hydrochlorides were hydrogenated under similar conditions except that the catalyst was platinum oxide. Four moles of hy-

drogen were absorbed, the first three within 15 minutes, the fourth in about two hours. The products were obtained as orange gums, which resisted all attempts to crystallize them.

Reaction of β -Diethylamino-*p*-nitropropiophenone and Phenylhydrazine.—A mixture of 5 g. of β -diethylamino-*p*-nitropropiophenone hydrochloride, 5 g. of phenylhydrazine and 2 g. of anhydrous sodium acetate in 50 ml. of ethanol was heated on the steam-bath for two hours. A dark gummy solid was collected by filtration and washed on the filter with water. After several recrystallizations from pyridine-methanol, 2.2 g. of dark red crystals was obtained, m.p. 152.0–152.5°. Analytical data are in agreement with those calculated for 3-(*p*-nitrophenyl)-1-phenylpyrazoline.

Anal. Calcd. for $C_{15}H_{13}N_3O_2$: C, 67.4; H, 4.9. Found: C, 67.6; H, 5.0.

RESEARCH DIVISION
BRISTOL LABORATORIES, INC.
SYRACUSE, NEW YORK

COMMUNICATIONS TO THE EDITOR

A LINK BETWEEN FATTY ACYL CoA DEHYDROGENASE AND CYTOCHROME *c*: A NEW FLAVIN ENZYME¹

Sir:

A green (G)¹ and yellow (Y)² flavoprotein catalyze the dehydrogenation of fatty acyl derivatives of CoA.³ A preparation of Y from pig liver showed four peaks on electrophoresis.⁴ The proteins constituting two of these peaks were identified as G⁵ and Y, respectively. Although these sampled components were immediately reducible by substrate, the reduced forms were not oxidizable by electron acceptors. Catalytic activity with indophenol³ could be restored when G or Y was supplemented with the additional protein components separated during electrophoresis. These latter components were not reducible by substrate nor did they catalyze the oxidation of substrate by indophenol. Only certain fractions of this factor (DRF)⁶ which mediates the reoxidation of reduced G and Y by indophenol are able to catalyze the interaction with Cyt as well. This Cyt reducing factor (CRF) was isolated from pig liver and was found to be a flavoprotein with the following characteristics: (1) extinction maxima at 270, 375 and 440 m μ , minima at 310 and 400 m μ ($E_{270}:E_{310}:E_{375}:E_{440} = 6.5:0.3:0.9:1.0$); (2) a characteristic shoulder at 450–460 m μ ; (3) a riboflavin content

(1) D. E. Green, S. Mii, H. R. Mahler and R. M. Bock, *J. Biol. Chem.*, **206**, 1 (1954); H. R. Mahler, *ibid.*, **206**, 13 (1954).

(2) H. Beinert and F. L. Crane, *Fed. Proc.*, **13**, 181 (1954); D. E. Green, *Biol. Revs.*, **29**, 330 (1954). The relationship of G or Y to a similar enzyme reported by W. Seubert and F. Lynen, *THIS JOURNAL*, **75**, 2787 (1953), cannot be evaluated on the basis of available data.

(3) CoA = coenzyme A; indophenol = 2,6-dichlorophenolindophenol; Cyt = cytochrome *c*.

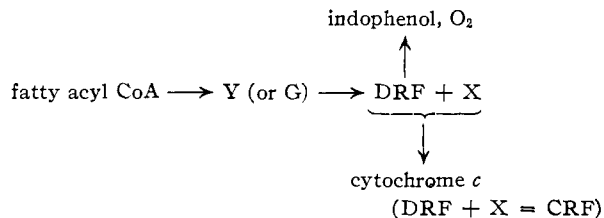
(4) Kindly carried out for us by Dr. R. M. Bock.

(5) The amount of G present was about 5% of that of Y.

(6) DRF = dye reducing factor; CRF = cytochrome *c* reducing factor. DRF and CRF also catalyze the auto-oxidation of G or Y; DRF and CRF are heat labile.

of 0.45%; (4) the released flavin was identified as FAD by its spectrum and by paper chromatography; it replaces FAD quantitatively with *d*-amino acid apo δ oxidase; (5) the flavin is not reducible by a fatty acyl CoA except in presence of catalytic mounts of Y (25% of $E_{440m\mu}$ remaining). The purest CRF fractions are likewise the most active DRF preparations, the ratio of CRF to DRF activity being about 1. Such preparations lose CRF activity within a few days without decline of DRF activity or perceptible change in the spectrum. This relationship of CRF to DRF is reminiscent of that between Cyt reductase and diaphorase.⁷ CRF seems to be a more complex form of DRF, which retains only DRF activity after some degradative process. So far a metal could not be implicated in this phenomenon.

No metal has been found in Y in an amount comparable to that of copper in G¹. Nonetheless, G requires CRF for optimal interaction with Cyt. The following scheme illustrates the path of electrons, indicated by the arrows, as it appears now in view of the reported work:



INSTITUTE FOR ENZYME RESEARCH
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

FREDERICK L. CRANE⁸
HELMUT BEINERT

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(7) H. R. Mahler and D. G. Elowe, *THIS JOURNAL*, **75**, 5769 (1953).

(8) Postdoctoral trainee of the National Heart Institute, National Institutes of Health.