intense Soret absorption of hemoglobin (400–450 nm) is proportional to the ratio of the concentration of the fluorescent compound to that of heme. When the fluorophore is present in the erythrocyte, as in the case for zinc protoporphyrin or FEP, the emission intensity is independent of hematocrit. Although we have tried to point out these features of hematofluorometry (4–6), they have been consistently overlooked. One reason for this is that most commercial hematofluorometers are "calibrated" to read ZPP or FEP in micromgs per deciliter of blood, the unit required by regulations of the Center for Disease Control concerning lead intoxication screening (7). This unit was chosen because FEP was customarily determined by extraction methods. While the "natural" measurement made by hematofluorometry is the porphyrin/heme ratio, manufacturers convert this ratio to a porphyrin concentration by multiplying by a "statistical" hematocrit value of 35 and 42 for children and adults, respectively (8).

Hematofluorometer manufacturers could easily calibrate instruments to read the FEP/heme ratio, micromgs of ZPP or FEP per gram of hemoglobin, or micromoles of ZPP or FEP per mole of heme, which would be both more accurate and more meaningful. In fact, some commercial instruments in use in Europe are calibrated in these units.

We also wish to point out that commercial hematofluorometers with appropriate emission filters detect both zinc protoporphyrin and metal-free protoporphyrin in blood, but possibly with different efficiencies. In our prototype instrument, calibrated for zinc protoporphyrin, the "recovery" of free protoporphyrin is 93%.

About 75% of the laboratories now taking part in the Center for Disease Control FEP proficiency program are using hematofluorometers (9). Thus it no longer seems justified to retain the present units, and a shift to some unit which reflects the porphyrin/heme ratio would appear appropriate.

References
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Angelo A. Lamola
Josef Eisinger
William E. Blumberg

Bell Laboratories
Murray Hill, NJ 07974

Substrate Specificity of Cholesterol Oxidase

To the Editor:

We have developed enzymic polarographic methods for the direct and rapid micro-scale measurement of glucose, galactose, and plant and animal sterols (1–3), and want to comment on the recent paper by Kritchevsky and Tepper (4) and certain other reports in the literature (5, 6) concerning the specificity of cholesterol oxidase (EC 1.1.3.6). Our method depends upon the quantitative measurement of cholesterol oxide by an oxygen-insensitive membrane-protected platinum electrode, polarized as an anode, mounted in a stirred, O2-equilibrated, thermostated (50 °C) cuvet. The injected sample is diluted about 13-fold with a special buffer in the cuvet, after which, in the case of cholesterol measurements, it is simultaneously exposed to cholesterol esterase (EC 3.1.1.13) and cholesterol oxidase.

We are testing cholesterol oxidases derived from Nocardia, Brevibacterium, and Actinomycetes, as obtained from several sources. We are also examining some steroid substrates, to elucidate the specificity of these various enzyme preparations. Other factors such as dispersion of the sterol, solvent and solute interactions, and the like are also being studied.

The following steroids gave responses quantitatively equivalent to cholesterol:
(a) 5α-androstan-3β-ol-17-one (epiandrosterone),
(b) 5-androsten-3β-ol-17-one (dehydroepiandrosterone),
(c) 5-androsten-17α-methyl-3β,17β-diol,
(d) 5-androsten-3β-ol, (e) campesterol,
(f) 5α-cholestan-3β-ol,
(g) 5-cholic acid-3β-ol,
(h) bisnorcholeic acid, (i) 5β-pregnene-3β,20α-diol, (j) 5α-pregnene-3β,20β-diol, (k) 5-pregnen-3β-hydroxy-16α,17α-epoxy-20-one, (l) stilbestrol, (m) stigmasterol, (n) ergosta-5,7,22-trien-3β-ol (ergosterol), (o) dehydroepiandrosterone, and (p) 5α-androstan-3β-ol-17-one O-carboxymethylxime. Interestingly, 1 is oxidized only by the cholesterol oxidase obtained from Ferrnco Biochemicals Inc., but at a slower rate than cholesterol and less than stoichiometrically, but none of the cholesterol oxidase preparations generated peroxide from 5β-androstan-3β-ol.

In summary, then, we have found, contrary to Kritchevsky and Tepper, that c is a substrate for cholesterol oxidase and, contrary to Richmond (6) but in agreement with Allain et al. (5) and Flegg (7), that f is oxidized by cholesterol oxidase. Our studies are still in progress, but it appears at present that the specificity of cholesterol oxidases towards steroids lies in the A and B rings of the cholestane configuration and that substituents in the 17-position have little, if any, effect.

References

Leeland C. Clark, Jr.

Elland & Bethesda Aves.
Cincinnati, OH 45229

Thomas A. Grooms

Yellow Springs Instrument Co.

Yellow Springs, OH 45387