Table 1. Effects of Isocaloric Egg Substitution in Diet of Eight Men, Mean Values for Plasma at Four-Week Periods

		HDL-Cholesterol [a]		LCAT [b]		Cholesterol [a]		Triglyceride [a]	
	Age	Control	Diet	Control	Diet	Control	Diet	Control	Diet
	43	41	42	4.0	5.7	206	213	221	242
	41	59	61	4.0	5.6	178	168	61	53
	53	43	42	3.8	3.8	282	304	243	221
	35	53	51	5.2	6.4	207	207	156	164
	50	75	74	3.8	4.6	205	222	37	48
	57	72	79	2.5	2.9	250	301	118	88
	30	54	54	4.5	4.8	179	195	115	96
	63	53	50	3.5	3.3	235	249	150	172
Mean	46.5	56.3	56.6	3.9	4.6	217.7	232.4	137.6	135.5

[a] Values expressed in milligrams/deciliter.
[b] Values are expressed as percent cholesterol esterified per hour.

eight subjects studied in the interplay of absorption, synthesis, and re-excretion. More recently, Nestel and Poyser (*3*) reported that the sum of changes in synthesis and re-excretion of cholesterol generally equaled the change in absorption when men were fed 500 mg of cholesterol from powdered egg yolk, although some of these subjects showed increases in plasma cholesterol in spite of the compensatory changes.

Our interest in this problem has been stimulated by the finding in our laboratories of an HDL-cholesterol concentration of 950 mg/liter in the plasma of a 63-year-old man who has consumed six eggs daily for several years. To further evaluate the significance of this finding, we set a preliminary protocol for male subjects in the 30–60 year age group. These individuals recorded their normal diet for four weeks, during which time blood was sampled, while the person was fasting, every seven days. Four eggs were then added isocalorically to the individual's daily dietary regime, and fasting blood samples were again collected every seven days for 28 days

The specimens were analyzed for cholesterol, triglyceride and HDL-cholesterol by the Lipid Research Clinic methods (*4*) and for lecithin:cholesterol acyltransferase (EC 2.3.1.43) by the method of Stokke and Norum (*5*).

The data shown in Table 1, the means for each subject from the control and experimental periods, indicate that during the experimental period there was no significant change in either HDL-cholesterol or triglyceride concentrations compared with control periods. There is an apparent increase in total cholesterol concentration. In agreement with other findings (*2, 3*), this varies from subject to subject.

Lecithin: cholesterol acyltransferase activity increased in six of eight subjects. Those subjects showing the greater increase in this activity had no substantial change in plasma cholesterol despite a daily challenge for one month of about 1100 mg of egg cholesterol. Those subjects showing minimal increases in the activity of this acyltransferase showed greater increases in plasma cholesterol during the diet period.

We know of no other report which indicates a direct relationship between cholesterol challenge in the diet and increased lecithin:cholesterol acyltransferase activity with the effect of maintaining plasma cholesterol equilibrium.

References

1. Mistry, P., Nicoll, A., Niehaus, C., and Christie, I., Cholesterol feeding revisited. *Circulation* **54**, II, 178 (1976). Abstract
2. Quintao, E., Grundy, S. M., and Ahrens, E. H., Effects of dietary cholesterol on the regulation of total body cholesterol in man. *J. Lipid Res.* **12**, 233 (1971).
3. Nestel, P., and Poyser, A., Changes in cholesterol synthesis and excretion when cholesterol intake is increased. *Metabolism* **25**, 1591 (1976).
4. Manual of Laboratory Operations. Lipid Research Clinics Program. DHEW Publication No. (NIH), U. S. Govt. Printing Office, Washington, DC (1974).
5. Stokke, K. T., and Norum, K. R., Determination of lecithin:cholesterol acyltransferase in human blood plasma. *Scand J. Clin. Lab. Invest.* **27**, 21 (1971).

Howard C. Elliott

Dept. of Pathol.
Baptist Med. Center
Birmingham, Ala. 35213

Hydroxy-Hemopyrrolenone, *not* Kryptopyrrole, in the Urine of Schizophrenics and Porphyrics

To the Editor:

For many years there has been growing evidence that some metabolite reacting with Ehrlich's reagent, and different from all well-known Ehrlich-reactors, is associated with psychiatric disorders in general and with psychoses or schizophrenia in particular. Many references to this work were given in the recent paper by Gendler, Duhan, and Rapoport (*1*). Their paper reported attempts to measure unoxidized kryptopyrrole and hemopyrrole, as such, in urine from normal persons and psychiatric patients. Neither of these pyrroles was found (*1*).

While the findings of Gendler et al. are interesting, I believe it is important to recognize that the metabolite referred to above and historically known as "mauve factor" or "natural kryptopyrrole" in *not* kryptopyrrole, but 2-hydroxyhemopyrrolene-5-one, as we made clear in 1975 and 1976 (*2*). More formally, this same molecular structure may be named 2-hydroxy-4-ethyl-2,3-dimethyl-Δ^3-pyrrolene-5-one, and it has been called α'-hydroxyhemopyrrole-α-lactam, HPL, HHPO, or, most recently (*3*), 3-ethyl-5-hydroxy-4,5-dimethyl-pyrrolin-2-one, EHDP. The relationships between this specific structure and those of kryptopyrrole and hemopyrrole can be seen in Figure 1. This *hydroxylated pyrrolenone* is the substance with the interesting clinical neuropsychiatric correlates.

In 1969 we reported the presence of oxygen in the structure of this metabolite (*4*); in 1974 we stated its structure was that of a hydroxypyrrolenone (*5*) subject to spontaneous adduct exchange resulting in multiple zone formation; in 1975 and 1976 (*2*) we reported the exact isomeric structure of that hydroxypyrrolenone. Consequently, it is several years since this metabolite has been thought of as kryptopyrrole, and from the beginning that term was used for brevity and to indicate the simplest possible form of the substance which would be expected to autoöxidize readily, yielding the multiple forms of adducts of the metabolite as it is found in urine extracts.

In terms of simple pyrroles *per se* in urine, I agree fully with the final conclusion of Gendler, Duhan and Rapoport: ". . . no evidence exists for the occurrence of hemopyrrole or kryptopyrrole in urine of either normal persons or schizophrenics." But this is *not* to deny

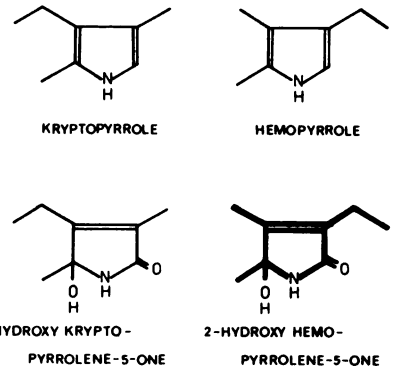

Fig. 1. Molecular structures of unoxidized kryptopyrrole, hemopyrrole, the unnatural isomer of the metabolite (hydroxykryptopyrrolenone), and *in bold outline*, the natural metabolite (hydroxyhemopyrrolenone)

the presence of the very closely related pyrrolic metabolite 2-hydroxyhemopyrrolene-5-one (HHPO) associated with schizophrenic and porphyric symptomatology. Indeed, the evidence for this metabolite and its clinical relevance has never been stronger. Thus, the metabolite has been synthesized (*6*) and new analytical techniques have been devised and applied (*2, 3, 5*), confirming the significant associations between this metabolite and schizophrenic symptoms (*7*) and hereditary hepatic porphyrias (*2, 3*). In addition, compounds which are isomeric with the metabolite have been shown to be active inducers of excessive porphyrin formation (*8, 9*). While tests of the porphyriogenicity of the metabolite itself are not yet complete, such activity can be expected, and would raise the likelihood that the metabolite is involved in the pathogenesis of acute intermittent porphyria (Irvine, unpublished). Consequently, the metabolite exists, and has interesting clinical correlates and a well-defined molecular structure.

There is no longer anything puzzling about the multiple-spotting tendency of this metabolite (*10*); neither is there any real question about its exact chemical structure (*2*). Because the metabolite belongs to the *hemo* series of pyrrole derivatives, it is not going to arise from kryptopyrrole, should perchance any of this pyrrole be produced in the body. While it is still conceivable that HHPO might arise from hemopyrrole as such (conceivably formed at least transitorily in the body) this would be excruciatingly difficult to establish because of the well-documented extreme tendency of hemopyrrole to auto-oxidize. It is much more plausible to suggest that HHPO arises from suitably constituted end-rings of bile pigments (including the urobilinogens). This would provide a pathway to HHPO without any intermediate hemopyrrole.

In summary, then, it is important to recognize that the metabolite once known as "mauve factor" or "natural kryptopyrrole" is specifically 2-hydroxy-4-ethyl-2,3-dimethyl-Δ^3-pyrrolene-5-one.

References

1. Gendler, P. L., Duhan, H. A., and Rapoport, H., Hemopyrrole and kryptopyrrole are absent from the urine of schizophrenics and normal persons. *Clin. Chem.* **24,** 230 (1978).

2. Irvine, D. G., and Wilson, D. L., Oxidized monopyrroles in porphyric disorders and related conditions. First International Porphyrin Meeting, Freiburg, 1975. In *Porphyrins in Human Diseases,* M. Doss, Ed., Karger, Basel, 1976, pp 217–224.

3. Granam, D. J. M., Brodie, M. J., Moore, M. R., and Goldberg, A., Quantitation of a urinary pyrrolic metabolite found in excess in acute intermittent porphyria. *Scott. Med. J.* **22,** 243 (1977).

4. Irvine, D. G., Bayne, W., Miyashita, H., and Majer, J. R., Identification of kryptopyrrole in human urine and its relation to psychosis. *Nature* **224,** 811 (1969).

5. Irvine, D. G., Kryptopyrrole and other monopyrroles in molecular neurobiology. *Int. Rev. Neurobiol.* **16,** 145 (1974).

6. Irvine, D. G., Synthesis, reactivity and toxicity of a pyrrolic metabolite associated with porphyrias. *Proc. Can. Fed. Biol. Soc.* **20,** 179 (1977).

7. Irvine, D. G., Clinical, EEG and biochemical correlates of hydroxyhemopyrrolenone excretion. *Proc. Sask. Psychiat. Res. Meet.* **22, 59 (1977).**

8. Durkó, I., Berek, I., and Huszák, I., Effects of kryptopyrrole or porphyrin synthesis in *Bacillus subtilis* 168. *Hoppe-Seyler's Z. Physiol. Chem.* **356,** 1679 (1975).

9. Graham, D. J. M., Moore, M. R., Thompson, G. G., and Goldberg, A., The effect of 4-ethyl-5-hydroxy-3,5-dimethyl-Δ^3-pyrrolin-2-one on porphyrin synthesis in the rat. *Biochem. Soc. Trans.* **4,** 1089 (1976).

10. Irvine, D. G., Autotransfer chromatography in the characterization of pyrroles: Chemistry of multiple-spot phenomena. *J. Chromatogr.* **123,** 69 (1976).

D. G. Irvine

Psychiatric Research Division
University Hospital
Saskatoon, Saskatchewan
Canada S7N 0W8

Higher Values for Ionized Calcium with a New Type of Electrode for Orion SS-20

To the Editor:

Since 1976 we have used the Orion SS-20 for routine determination of serum ionized calcium. Our reference range was 0.96–1.16 mmol/liter, based on serum samples from 110 inpatients and 30 outpatients without history or symptoms of disturbed calcium metabolism. This spring (1978) we had some trouble with unstable potential, which we could attribute to the electrode batch (IZ2). Simultaneously, the recorded ionized calcium values seemed to be under-estimated as compared with the reference range. We therefore asked Orion for a new electrode batch, which (IV1) arrived in June. The potential for this electrode was stable, but it gave considerably higher values for serum ionized calcium than IZ2. Because we have a great many follow-up patients whose treatment depends on results of analyses for ionized calcium, we had to establish the magnitude of this difference. We also had to change our reference range.

During three days, four series of samples were analyzed with both electrodes. The samples consisted of two series of samples sent for routine calcium analysis (n = 32) and two series (reference group, n = 30) of samples from healthy laboratory workers and outpatients with no known disturbance of calcium metabolism. All samples were taken in Vacutainer Tubes (Becton-Dickinson, Inc.) without addition, and serum was aspirated through the stopper for the analysis.

The relationship between the values for ionized calcium in serum for the two electrodes is shown in Figure 1. It is evident that the new electrode gives higher recordings than the old one. The difference between the recordings was 0.178 ± 0.014 (mean ± SEM, throughout) for the reference group and 0.184 ± 0.039 for all pairs of observations. The greater SEM for the latter group could be attributed to one of the routine series containing many sera with pathological values (14/20 beyond the reference range).

Our old reference range took into account that inpatients have a significantly higher (1.067 ± 0.0046) serum ionized calcium than do outpatients (1.039 ± 0.0074)—($t = 3.22, P < 0.005$). We assumed that our reference group and our outpatient group were randomly

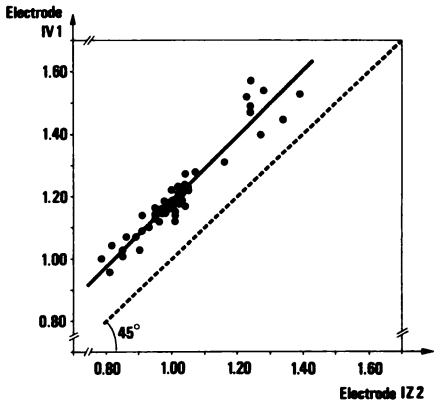

Fig. 1. Relation between recordings of serum ionized calcium with the new type of electrode (IV1) and the old type (IZ2)
The regression line (—) corresponds to the equation: $y = 1.046x + 0.137$. Correlation coefficient, 0.958