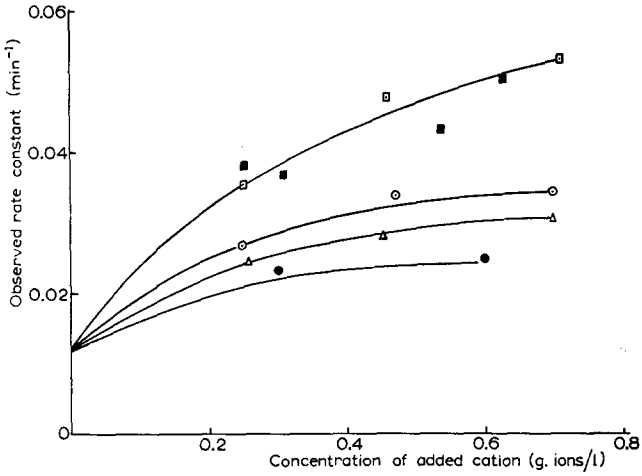

Fig. 2. The effect of cations on the reactivation by isonitrosoacetone (0.004 M) at pH 7.8 and 25° of erythrocyte ChE inhibited with TEPP. □ KCl, ■ K_2SO_4, ○ NaCl, ● Na_2SO_4, △ NH_4Cl.

Although reactivation by isonitrosoacetone is also accelerated by complex formation[2], the formation of this complex does not involve the anionic site on the enzyme, in which case the slowing down of reactivation due to cations, found with pyridine-2-aldoxime methiodide, would not necessarily be expected. In fact, Fig. 2 shows that cations, instead of retarding reactivation by isonitrosoacetone, markedly accelerate it. A similar acceleration due to cations is found when picolinhydroxamic acid is used as a reactivator. This difference between positively charged and uncharged reactivators is consistent with the belief[2] that they form complexes at different sites on the phosphorylated enzyme. However, no satisfactory explanation can be offered why the rate of reactivation by uncharged reactivators should be greatly increased in the presence of electrolytes.

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Isolation of 2-methoxyoestrone from the urine of pregnant women

KRAYCHY AND GALLAGHER[1] have recently reported the isolation of 2-methoxyoestrone from the urine of human subjects following the administration of oestradiol-17β-16 ^{14}C. This finding has been confirmed by ENGEL *et al.*[2]. In the course of our own studies on urinary oestrogen metabolites, the same compound has now been isolated from the urine of pregnant women, and observations of some interest have been made concerning its behaviour in the Kober reaction.

Preliminary experiments were carried out on synthetic 2-methoxyoestrone generously supplied by Dr. T. F. GALLAGHER of the Sloan-Kettering Institute, New York.

In the Kober reaction carried out by the procedure of BROWN[3] as modified by BAULD[4], using the latter's "oestriol reagent", 2-methoxyoestrone gave an immediate orange-pink colour in the first stage of the reaction which changed to red after 20 min heating. In the second stage of the reaction the colour changed to a purple-pink, which showed a rather flat absorption maximum between 545 and 550 mμ (*cf.* oestriol and oestrone, max. at 512.5 mμ).

Good separation of 2-methoxyoestrone from oestrone was achieved by chromatography on a Celite column using the solvent system 70% (v/v) methanol in water/30% (v/v) benzene in *n*-hexane.

The starting material for the isolation was ketonic-phenolic material from 700 l of enzymically hydrolysed late pregnancy urine prepared as described by MARRIAN *et al.*[5]. The fraction of this containing oestrone and less "polar" ketonic phenols had been previously separated on a Celite column in the system 70% (v/v) methanol in water/20% (v/v) *n*-hexane in benzene. This fraction was chromatographed on Celite using the system 70% (v/v) methanol in water/30% (v/v) benzene in *n*-hexane. Those portions of the eluate which gave a Kober reaction suggestive of the presence of 2-methoxyoestrone were combined and yielded 94 mg of a brown oil. On leaching this with a small volume of acetone at —20° about 10 mg of a white crystalline substance was obtained. This gave a negative Kober reaction and was not further investigated. The material soluble in cold acetone was chromatographed on Al_2O_3 (acid washed; activated by heating at 140°) using benzene-hexane, benzene and acetone-benzene for elution. The fractions richest in the 2-methoxyoestrone-like Kober chromogen were combined and recrystallized twice from methanol at —20°, when 2.1 mg of a white crystalline product, m.p. 183–184° (uncorr.), was obtained. A further 2.1 mg of material, m.p. 182–183°, was obtained from the mother liquors. The infrared spectrum on a sample of the product was kindly determined by Dr. T. F. GALLAGHER who reported that it was "identical in all respects with that of 2-methoxyoestrone".

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Studies of hemins a_1 and a_2*

Several studies[1–11] have shown that it is possible to cleave the hemin from cytochrome *a* components of muscle and extract it into organic solvents. There was, however, no complete agreement on the properties of the compounds isolated. NEGELEIN[7] indicated that the cryptohemin he had isolated, the reduced pyridine hemochromogen of which had absorption bands at 582 and 533 mμ, was an artifact derived from protohemin. In a reinvestigation and extension of these studies, ROCHE AND BENEVENT[8] concluded that a pigment with absorption maxima in the same positions as those of NEGELEIN's cryptohemin, is the compound first extracted from heart muscle, and further that this compound can give rise during isolation to a hemin with a single absorption band in the visible region at 587 mμ.

Recent work[9, 10, 11] has positively identified a hemin with absorption peaks at 430 and 587 mμ as a porphyrin compound which could be derived from cytochromes *a* and a_3. LEMBERG and coworkers[12, 13, 14] also investigated the cryptohemin and concluded that is was not derived from protohemin, but, because of the low concentration in which it was found, decided that it was not the prosthetic group of either cytochrome *a* or a_3. MORRISON AND STOTZ[10] were able to isolate two hemins from a purified cyotchrome *a* and a_3 preparation. These hemins were labeled a_1 and a_2. In our earlier reports it was indicated that the spectra of these two compounds were similar. In the present study, with the aid of a new paper chromatographic method[15], it became evident that there was cross-contamination of the two components on the column. The new technique permitted us to

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