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".

The authors are grateful to the Medical Research Council for a grant from which the expenses of this work were defrayed.

K. H. LOKE
G. F. MARRIAN

Department of Biochemistry, University of Edinburgh (Scotland)

[1] S. KRAYCHY AND T. F. GALLAGHER, *J. Am. Chem. Soc.*, 79 (1957) 754.
[2] L. L. ENGEL, B. BAGGETT AND P. CARTER, *Endocrinology*, 61 (1957) 113.
[3] J. B. BROWN, *J. Endocrinol.*, 8 (1952) 196.
[4] W. S. BAULD, *Biochem. J.*, 56 (1954) 426.
[5] G. F. MARRIAN, E. J. D. WATSON AND M. PANATTONI, *Biochem. J.*, 65 (1957) 12.

Received October 29th, 1957

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

* This research was supported in part by the Life Insurance Medical Fund, Grant No. G 56–45, and by the National Heart Institute, U.S. Public Health Service, Grant No. 1322.

pilot the column procedure, and by rechromatography ultimately to obtain the pure individual components. Thus it has become possible to demonstrate distinct differences in their spectral properties.

The hemins were extracted from heart muscle as described by CONNELLY, MORRISON AND STOTZ[16], and were purified by the column chromatographic procedure[10]. Spectra of oxidized and reduced pyridine hemochromogens were obtained with a Beckman DU spectrophotometer employing a solvent which consisted of 1 ml pyridine and 2 ml 0.05 N NaOH. The hemins were converted to the porphyrins by a modification of the procedure of MORELL AND STEWART[17]. To 1 μmole hemin dissolved in 10 ml glacial acetic acid, 0.3 ml iron reagent was added under CO_2 or N_2. The solution was allowed to stand for 5 to 10 min, poured into an equal volume of ether, and then layered over the same volume of saturated sodium acetate. The iron reagent was made up of 3.0 g $FeSO_4 \cdot 7H_2O$ dissolved in 15 ml 0.23 N HCl.

Fig. 1 shows the spectra of the oxidized and reduced pyridine hemochromogens of hemins a_1 and a_2. The reduced hemin a_1 has an absorption peak at 582 mμ, while a_2 has a maximum absorption at 587 mμ. The difference between the two hemins is not as great in the Soret region, only 2 mμ separating their maxima at 432 and 430 mμ. It may be noted that the spectrum of hemin a_1 has a small β-peak at 533 mμ, which is missing in the hemin a_2 spectrum. Table I shows a comparison of the spectra of hemins a_1 and a_2 and the porphyrins derived from these hemins, with the data that LEMBERG and his group have reported [12, 13]. The similarities are apparent.

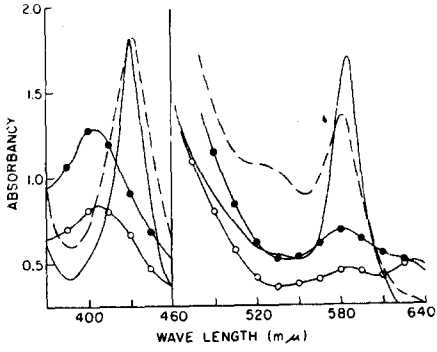

Fig. 1. Spectra of pyridine hemochromogens of hemin a_1 oxidized —○—, reduced -----; hemin a_2 oxidized —●—, reduced ——.

Recently, LUNDEGARDH[18] observed that on treatment of a heart muscle oxidase preparation with pyridine and alkali there occurs a peak at 583–584 mμ with a low extinction, and this peak shifts with time to 586–588 mμ with an increase in the extinction. Earlier, MORRISON AND STOTZ[19] studied the rate of release of the hemin from a purified cytochrome oxidase preparation. It was noted that the hemins were released from the proteins at two considerably different rates. Chromatography showed that it was hemin a_1 which is more rapidly extracted than hemin a_2. It seems possible that hemin a_1 (α-peak 582 mμ) is the most rapidly extracted by the procedure employed by LUNDEGARDH as well, and that later release of hemin a_2 (α-peak 587 mμ) might cause the shift in the spectrum toward the higher wave length.

The data in Table I indicate that hemin a_1 is closely related in its spectral properties to the cryptohemin a of LEMBERG[13]. Although LEMBERG has considered his cryptohemin a to be an artifact from the preparative procedure, it is interesting to note that other investigators have reported the presence of a β-band in hemin a preparations similar in position to that occurring in hemin a_1[9, 20]. LEMBERG [21] accounts for the presence of the β-bands on the basis of solvents employed for the spectral work. We have not been able to observe the presence of these bands in purified hemin a_2 preparations, but consider that this band is an indication of the presence of hemin a_1.

TABLE I

COMPARISON OF SPECTRAL PROPERTIES OF HEMIN a TYPE COMPOUNDS

Compound	*Pyridine hemochromogen mμ*		*Pyridine hemochromogen of oxime mμ*		*Porphyrin mμ*
Hemin a_1	582	535	562	532	638, 583, 558, 513, 418
Hemin a_2	587	—	575	530	646, 583, 558, 516, 412
Cryptohemin a*	582	533	562	533	642, 584, 559, 513, 421
Hemin a**	587	—	570	533	647, 583, 558, 517, 412

* LEMBERG AND FALK[12].
** LEMBERG[13].

From the data presented here, and our earlier report[10] which gave no evidence of interconversion of the two hemins *a*, we are not inclined to discard LEMBERG's cryptohemin *a*, which appears to be identical with our hemin a_1, as artifact.

Dept. of Biochemistry, Medical Center, University of Rochester, Rochester, N.Y. (U.S.A.)

MARTIN MORRISON
JERALD CONNELLY
ELMER STOTZ

1 E. NEGELEIN, *Biochem. Z.*, 248 (1932) 243.
2 E. NEGELEIN, *Biochem. Z.*, 266 (1933) 412.
3 W. A. RAWLINSON AND J. H. HALE, *Biochem. J.*, 45 (1949) 247.
4 J. E. FALK AND C. RIMINGTON, *Biochem. J.*, 51 (1952) 36.
5 O. WARBURG, H. GEWITZ AND W. VOLKER, *Z. Naturforsch*, 10 (1955) 541.
6 R. LEMBERG, B. BLOOMFIELD, P. CAIGER AND W. H. LOCKWOOD, *Australian J. Exptl. Biol. Med. Sci.*, 33 (1955) 435.
7 E. NEGELEIN, *Biochem. Z.*, 250 (1932) 577.
8 J. ROCHE AND M. T. BENEVENT, *Bull. soc. chim. biol.*, 18 (1936) 1650.
9 P. PERSON, W. W. WAINIO AND B. EICHEL, *J. Biol. Chem.*, 202 (1953) 369.
10 M. MORRISON AND E. STOTZ, *J. Biol. Chem.*, 213 (1955) 373.
11 H. DANNENBERG AND M. KIESE, *Biochem. Z.*, 322 (1952) 395.
12 R. LEMBERG AND J. E. FALK, *Biochem. J.*, 49 (1951) 674.
13 R. LEMBERG, *Nature*, 172 (1953) 619.
14 R. LEMBERG AND J. PARKER, *Australian J. Exptl. Biol. Med. Sci.*, 33 (1955) 451.
15 M. MORRISON AND E. STOTZ, *J. Biol. Chem.*, 228 (1957) 123.
16 J. CONNELLY, M. MORRISON AND E. STOTZ, *Federation Proc.*, 16 (1957) 167.
17 D. B. MORELL AND M. STEWART, *Australian J. Exptl. Biol.*, 34 (1956) 211.
18 H. LUNDEGARDH, *Biochim. Biophys. Acta*, 25 (1957) 1.
19 E. STOTZ, M. MORRISON AND G. MARINETTI, in O. H. GAEBLER, *Enzymes: Units of Biological Structure and Function*, Academic Press Inc., New York, 1956.
20 M. KIESE AND H. KURZ, *Biochem. Z.*, 325 (1954) 299.
21 R. LEMBERG AND M. STEWART, *Australian J. Exptl. Biol. Med. Sci.*, 33 (1955) 451.

Received October 5th, 1957

Preliminary Notes

Latent ribonuclease activity in a ribonucleoprotein

In an account of an electrophoretic investigation of a ribonucleoprotein preparation obtained from *Escherichia coli* it was reported that exposure of the nucleoprotein to high concentrations of urea effected a separation of protein and ribonucleic acid (RNA), presumably by the rupture of hydrogen bonds between the two moieties[1]. The present communication contains a description of a second event which takes place in the same system, namely, the degradation of the RNA. This degradation appears to be the result of an enzymic process initiated by the action of urea.

It was initially observed that treatment of the nucleoprotein with 4 *M* or 7–8 *M* urea caused the appearance of two electrophoretically distinct ultraviolet-absorbing components, one migrating with the velocity of free RNA, and the other more slowly[1]. The slow component showed a mobility in starch, at pH 8.4, similar to that of a mixture of ribonucleotides. Eluted from the starch block, it was found to contain organically bound phosphorus. It may be noted that polymerized nucleic acid has previously been shown to have a higher electrophoretic mobility than nucleotides at pH 8.6[2].

When the duration of the urea treatment was held to a minimum (30 min before the start of electrophoresis, in these experiments), the slow component comprised only a small part of the ultraviolet-absorbing material. With the passage of time, however, the slow component increased in quantity, while the fast component diminished and eventually disappeared, indicating a progressive degradation of RNA to smaller fragments.

The degradation was then investigated by following the appearance of acid-soluble ultraviolet-absorbing material at 0–3°. In the absence of urea, nucleoprotein solutions were stable in the cold for at least one week, and precipitation by 0.1 *M* $HClO_4$ was complete (98–99%). The addition of urea initiated a release of acid-soluble material which continued until all of the RNA was degraded.