HEME AND TISSUE IRON.

By M. L. ANSON and A. E. MIRSKY.

(From the Biophysical Laboratories of the Cancer Commission, Harvard University, Boston.)

(Accepted for publication, October 4, 1928.)

There are a variety of heavy metal compounds in living cells. These are supposed to be involved in the catalysis of biological oxidations. It is by combination with these heavy metal compounds that the respiratory poisons cyanide, hydrogen sulfide, and carbon monoxide are supposed to act.* Little has been known about the precise nature of these substances except that one cannot get a test for free ionic iron. Yet the properties of an iron compound, depend very much on the nature of the complex of which the iron is a part. Thus the iron in hemoglobin has not the properties of the iron in ferric chloride. Over 40 years ago, however, MacMunn (10) observed in a great variety of animal tissues a pigment complex related to heme, the iron pyrrol part of hemoglobin. Recently Keilin (6) has shown that this complex, which he calls cytochrome, is present in the aerobic tissues of both plants and animals generally and that it consists of a mixture of pigments allied to hemochromogen. The components of cytochrome are not identical with the hemochromogen prepared from hemoglobin. There are likewise other naturally occurring pigments, chlorocruorin, helicorubin, and actineohematin, which either are or can be converted into pigments similar to but not identical with the hemochromogen from hemoglobin.

The old view of hemochromogen was that it is simply reduced heme, the iron pyrrol complex of hemoglobin. On the basis of this view the various pigments which have been mentioned all contain different iron pyrrol complexes since they differ from each other and from the

*The reactions between the heme pigments and the specific inhibitors of respiration will be discussed in a later paper.

hemochromogen of hemoglobin. The writers (1, 3) have shown, however, that every hemochromogen consists of heme joined to some nitrogenous substance. The possibility thus arises that the various different hemochromogen-like pigments contain the same heme joined to different nitrogenous substances. When the unknown nitrogenous substances of helicorubin and actineohematin were replaced with pyridine or ammonia the resulting hemochromogens were identical spectroscopically with the pyridine or ammonia hemochromogens prepared from the heme of hemoglobin. So we concluded (2) that helicorubin and actineohematin contain heme. By the same method Fox (5) later showed that the iron pyrrol complex of chlorocruorin is different from heme. The addition of pyridine to yeast produced typical pyridine hemochromogen (2). This led us to suppose that cytochrome itself contained heme. But Keilin (7) has since presented evidence that there is in yeast in addition to cytochrome large amounts of invisible heme. Our pyridine hemochromogen may have come solely from this heme and not from cytochrome. This leaves the precise nature of the iron pyrrol nuclei of cytochrome and their relation to heme an open question. Nevertheless, the experiments based on the new view of the composition of hemochromogen permitted the identification of the heme of hemoglobin in aerobic tissues generally and they showed that in the study of the naturally occurring iron pyrrol pigments the ability of iron pyrrol groups to combine with nitrogenous substances must be taken into consideration. The significance of these results is emphasized by the recent experiments of Kuhn and Brann (9) and of Krebs (8) which show that the catalytic powers of heme are greatly changed when the heme is slightly altered or when it is combined with a nitrogenous substance.

The demonstration that heme itself is present in all animals makes less surprising the extraordinarily haphazard distribution of the hemoglobins. It would be interesting to know whether other substances containing metal pyrrol groups such as hemocyanin and chlorocruorin have, like the heme pigments, some ancestor common to all animals.

The fact that heme is universally distributed raises the question of how much of the iron of organisms is in the form of heme and how much in the form of still unknown iron compounds. In our original paper (2) we stated that we were attempting to answer this question

by parallel total iron and heme analyses, the heme being extracted with pyridine and estimated as pyridine hemochromogen. Our results have hitherto not been published because the validity of such a technique has become doubtful. In the meantime, however, several investigators (4, 11) have carried out heme estimations using essentially the same procedure we suggested. And von Euler and Fink (4) have come to the conclusion that, in the yeast they had, only about one 1/160 of the total iron is in the form of heme.

If pyridine and a reducer are added to yeast and the mixture first shaken and then centrifuged there is obtained on top a clear pyridine solution and on bottom a layer of yeast. When examined spectroscopically both these layers show hemochromogen-like bands. In the

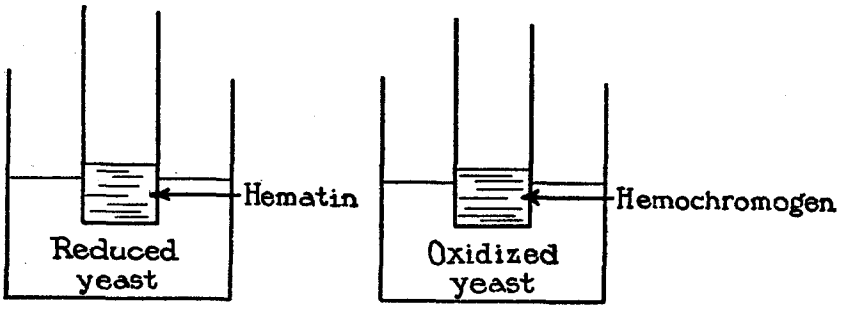

FIG. 1.

analyses of von Euler and Fink the pigment was examined in the clear pyridine solution and the assumption made that the concentration was the same in the yeast layer which was not examined. Such analyses are of little value in the absence of any precise information about the distribution of heme between the two layers. As a matter of fact, in the two cases we have studied, baker's yeast (Fleischmann's) and the wing muscles of bees, the assumption of equal distribution is not even approximately correct; mere inspection shows the concentration of hemochromogen to be much greater in the neglected yeast layer than in the clear pyridine solution.

Since we were unsuccessful in obtaining anything approaching a complete extraction of heme we decided to estimate the hemochromogen by comparing the density of its α band in the complete yeast-

pyridine mixture with the density of the α band of a series of known solutions of pyridine hemochromogen. In pure hemochromogen solutions whose concentrations are 10 per cent different one can readily detect the difference in the intensity of the bands. The hemochromogen from hemoglobin can be substituted for pyridine hemochromogen. If the heme contents of the solution of these two hemochromogens are the same, the intensity of the α bands are about the same too. In these experiments there were added to 4 gm. of moist yeast 1.5 cc. of 1/5 N NaOH and 1 cc. of pyridine.

It is necessary to compensate for the absorption of the yeast and its pigments. That is done as shown in the figure. By "oxidized yeast" (*cf.* Fig. 1) is meant yeast shaken with air for 5 minutes, which procedure causes the disappearance of the hemochromogen bands.

This method of analyses gives the result that 40 $\pm$ 10 per cent of the total iron of baker's yeast is in the form of heme. The total iron is 0.07 mg. per gm. dry weight.

One cannot tell how much of the iron of yeast has been introduced as an impurity during the commercial preparation of the yeast. This difficulty is avoided when the wing muscles of bees are used. Furthermore, the great activity of these muscles—they can contract several hundred times a second—is associated with an iron content even 2.8 times as great as that of yeast, namely, 0.196 mg. per gm. dry weight. Despite this great difference in the total iron we again found about 40 per cent of the iron to be in the form of heme.

The muscles were removed from frozen bees by the ingenious technique devised by Keilin (6). Care was taken not to introduce extraneous iron and to make the removal of the muscles as uniform as possible. Batches of the muscles from ten bees gave uniform analytical results.

The great weakness of these analyses lies in the use of pyridine hemochromogen as a standard. As Keilin (7) has pointed out, the hemochromogen-like substances obtained from cytochrome itself when pyridine is added may not be identical with pyridine hemochromogen. Furthermore, the α band of the hemochromogen seen in the muscle tissue obtained on centrifuging the muscle-pyridine mixture is not in precisely the same position as the α band of pyridine hemochromogen. These uncertainties render doubtful the estimations of heme based on the pyridine technique in any of its forms.

We hope, nevertheless, that the discussion and experiments which have been presented bring out some of the difficulties which have to be faced, and that they make clear, at least, that there is at present no adequate experimental basis for the view that only a very small part of tissue iron is in the form of heme. The evidence given by the type of procedure described in this paper points to heme being an important form of tissue iron.

The pyridine extract of yeast contains in addition to a hemochromogen similar to pyridine hemochromogen another hemochromogen-like pigment (2). Whether this second pigment contains an iron pyrrol complex is not known. There is the possibility that some and perhaps all of the iron not in the form of heme may exist in other iron pyrrol complexes.

SUMMARY.

1. A method is described for estimating the heme in yeast and bees' muscles as pyridine hemochromogen.

2. The difficulties of the method are discussed.

3. The heme as given by the pyridine method is responsible for about 40 per cent of the total iron.

REFERENCES.

1. Anson, M. L., and Mirsky, A. E., *J. Physiol.*, 1925, lx, 50.
2. Anson, M. L., and Mirsky, A. E., *J. Physiol.*, 1925, lx, 161.
3. Anson, M. L., and Mirsky, A. E., *J. Gen. Physiol.*, 1928–29, xii, 273.
4. von Euler, H., and Fink, H., *Z. physiol. Chem.*, 1927, cxlii, 272.
5. Fox, H. M., *Proc. Roy. Soc. London, Series B*, 1926, xcix, 199.
6. Keilin, D., *Proc. Roy. Soc. London, Series B*, 1925, xcviii, 312.
7. Keilin, D., *Proc. Roy. Soc. London, Series B*, 1926, c, 129.
8. Krebs, H. A., *Biochem. Z.*, 1928, cxciii, 347.
9. Kuhn, R., and Brann, L., *Z. Physiol. Chem.*, 1927, clxviii, 27.
10. MacMunn, C. A., *Phil. Tr. Roy. Soc. London*, 1886, clxxvii, 267.
11. Treibs, A., *Z. physiol. Chem.*, 1927, clxviii, 68.