dine. In both cases, the colour density is greater when haemoglobin is treated with the organic base than when an equivalent amount of haemin is treated with the organic base. The extinction coefficient of the pyridine haemochromogen from haemin is almost equal to that of globin haemochromogen whilst those of the pyridine haemochromogens from haemoglobin and blood are considerably higher. This suggests again that we are dealing with a substance which is a mixed or 'double' globin+pyridine haemochromogen. This is further suggested by the difference in the position of the absorption bands, those of the blood pyridine substance being between those of the other two. Drabkin & Austin (1935) have also noted a difference in the positions of the bands of haemin and haemoglobin pyridine haemochromogens. The difference between the pyridine haematins from haemin and haemoglobin respectively is consistent with the idea of a mixed haematin being obtained with haemoglobin.

Two explanations may account for the fact that the absorption bands of the purified haemoglobin derivative are lower than those of the corresponding blood derivative: (1) the presence of substances other than haemoglobin in blood; (2) degradation of the globin part of the haemoglobin molecule produced during the purification. The first

explanation seems less likely, since the positions of the absorption bands in the case of the blood are independent of its source, identical figures being obtained whether the blood is from normal individuals or anaemic patients. The position of the absorption bands is also identical for whole blood and washed cells.

SUMMARY

1. The colours given by solutions of several haematins and haemochromogens prepared from haemoglobin and from haemin are compared.

2. The results favour the theory that a conjugated protein is formed when blood is dissolved in 0.1N-NaOH, pyridine or diethylamine, with the haematin in the oxidized state.

3. The haematins formed by haemoglobin when treated with diethylamine and with pyridine, and the haemochromogen it forms with pyridine, appear to be different from those formed by haemin, and may be mixed compounds containing globin as well as the organic base.

4. The pyridine haemochromogen from purified haemoglobin differs slightly in the position of its spectral bands from that formed from blood.

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REFERENCES

- Anson, M. L. & Mirsky, A. E. (1930). *Physiol. Rev.* **10**, 506.
 Clegg, J. W. & King, E. J. (1942). *Brit. med. J.* **2**, 329.
 Delory, G. E. (1943). *Analyst*, **68**, 5.
 Drabkin, D. L. & Austin, J. H. (1935). *J. biol. Chem.* **112**, 89.
 Keilin, D. (1926). *Proc. Roy. Soc. B*, **100**, 129.
 King, E. J. (1942). *Lancet*, **1**, 511.

The Relationship between the Weight of Young Vitamin A-deficient Rats and the Increase in Weight after β -Carotene Administration

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Different workers hold different opinions on the correlation between a rat's body weight and its need for vitamin A. Greaves & Schmidt (1936) claim to have shown, on the basis of changes in the vaginal contents, that within the limits of age and body weights of the rats used by them, the vitamin A requirements are independent of these factors. They used cod-liver oil as their source of vitamin A. On the other hand, using the same criterion, but a slightly different dosing technique, Goss & Guilbert (1939) found that the rat's requirement of vitamin A was dependent on its body weight. The tests were carried out with β -carotene as well as cod-liver oil. Irving & Richards (1939) seemed to confirm this by using pathological changes in teeth and other organs

as their criteria. These last workers treated their rats prophylactically, from weaning, with solutions of vitamin A.

It was thought that some further light on this problem might be obtained by examining the weight response of rats deficient in vitamin A to the same daily dose of that factor. If, in fact, the rat's requirement of vitamin A is proportional to its body weight, then the increase in weight in response to a given dose of that vitamin, after the rat's stores have been depleted, should be in inverse proportion to its body weight at that time. For investigating this possibility, the results of vitamin A determinations carried out in this laboratory over many years were available.

EXPERIMENTAL

The rats, after weaning, were fed on a vitamin A-free diet (Coward, Key, Dyer & Morgan, 1930), until

in weight during that time determined. Scatter diagrams were plotted relating the increase in weight and the initial weight of each rat. The results were taken in strict chronological order for experiments carried out from 1937-9.

Fig. 1.

Fig. 2.

Fig. 1. Relation between the increase in weight of rats during 3 weeks' dosing with 1 i.u. of vitamin A (β -carotene) daily and the weight at which the rat was first given its dose (i.e. its weight when it had ceased to grow on a diet deficient in vitamin A) (200 males).

Fig. 2. Relation between the increase in weight of rats during 3 weeks' dosing with 1 i.u. of vitamin A (β -carotene) daily and the weight at which the rat was first given its dose (i.e. its weight when it had ceased to grow on a diet deficient in vitamin A) (200 females).

they were either just losing or had become steady in weight. This occurred at weights ranging from 50 to 118 g. for the males and from 50 to 110 g. for the females. Half of the rats used in each determination were then given a daily dose of one unit of the standard preparation of β -carotene. Their weights were recorded weekly for 3 weeks and their increase

RESULTS

Fig. 1 is plotted for 200 males and Fig. 2 for 200 females dosed during the same period.

It can be seen, without any statistical calculation, that there is no correlation between the initial body weight and the increase in weight after a dose of

vitamin A, and that there is no obvious difference between the sexes in this respect. Analysis of the results for a series of 50 animals taken chronologically from the two groups of 200 yielded a similar result, which showed that fluctuations in response over a long period of time were not masking any putative correlation between initial body weight and the increase in weight resulting from the administration of vitamin A.

SUMMARY

When a rat, receiving a vitamin A-deficient diet, is given β -carotene in oil, there is no correlation between the initial body weight and the increase in weight resulting from the administration of the vitamin.

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REFERENCES

Coward, K. H., Key, K. M., Dyer, F. J. & Morgan, B. G. E. (1930). <i>Biochem. J.</i> 24 , 1952.	Greaves, J. D. & Schmidt, C. L. A. (1936). <i>Amer. J. Physiol.</i> 116 , 456.
Goss, H. & Guilbert, H. R. (1939). <i>J. Nutrit.</i> 18 , 169.	Irving, J. T. & Richards, M. B. (1939). <i>Nature, Lond.</i> , 144 , 908.