SUMMARY

1. Evidence is presented which shows that Cu^+ is a much more effective agent than Cu^{++} in inhibiting urease, and that ascorbic acid acts as an inhibitor only in so far as it reduces $\text{Cu}^{++} \rightarrow \text{Cu}^+$. The experimental findings on which this evidence is based are as follows:

(a) Ascorbic acid does not inhibit urease when copper is absent, but does so when copper is present.

(b) The inhibition by Cu^{++} plus ascorbic acid is of the same magnitude as with Cu^+ alone. The inhibition produced by Cu^+ is not increased by the addition of ascorbic acid.

(c) Other reducing substances containing a dienol group and capable of reducing $\text{Cu}^{++} \rightarrow \text{Cu}^+$, e.g. hydroxytetronic, reductic and dihydroxymaleic acids, resemble ascorbic acid in their power to inactivate urease in the presence of copper.

(d) Urease inactivated either by Cu^{++} plus ascorbic acid or by Cu^+ alone may be reactivated by

H_2S , or by cysteine. It is postulated that the Cu^+ inactivates by forming a derivative with an $-\text{SH}$ group of the enzyme, and that substances like H_2S reactivate by removing the bound copper and regenerating this $-\text{SH}$ group.

2. Dehydroascorbic acid does not inhibit urease, even in the presence of copper. 2:3-Diketo-gulonic acid (produced by mutarotation when solutions of dehydroascorbic acid are left standing) inhibits only in the presence of copper, but to a less extent than does ascorbic acid.

3. Of the metallic salts tested, Cu^{++} and Au^{+++} alone had their toxicity increased by the addition of ascorbic acid. With Hg^{++} salts the toxicity was reduced by ascorbic acid.

I wish to thank Dr L. J. Harris for his valuable criticism and advice, and Mr B. J. Constable for his technical assistance.

The work described in this paper was carried out as part of a programme of the Food Investigation Board of the Department of Scientific and Industrial Research, by arrangement with the Medical Research Council.

REFERENCES

- Borsook, H., Davenport, H. W., Jeffreys, C. E. P. & Warner, R. C. (1937). *J. biol. Chem.* **117**, 237.
 Elson, L. A. (1943). *Nature, Lond.*, **152**, 49.
 Frankenthal, L. (1944). *Nature, Lond.*, **153**, 255.
 Giri, K. V. & Seshagiri Rao, P. (1944). *Nature, Lond.*, **153**, 253.
 Hellerman, L. (1937). *Physiol. Rev.* **17**, 454.
 Hellerman, L. (1939). *Cold Spr. Harb. Symp. Quant. Biol.* **7**, 165.
 Hellerman, L., Chinard, F. P. & Dietz, V. R. (1943). *J. biol. Chem.* **147**, 443.
 Hellerman, L., Perkins, M. E. & Clark, W. M. (1933). *Proc. nat. Acad. Sci., Wash.*, **19**, 855.
 Hopkins, F. G. (1929). *J. biol. Chem.* **84**, 269.
 Penney, J. R. & Zilva, S. S. (1943). *Biochem. J.* **37**, 39.
 Purr, A. (1934). *Biochem. J.* **28**, 1141.
 Quastel, J. H. (1932). *Biochem. J.* **26**, 1685.
 Quastel, J. H. (1943). *Nature, Lond.*, **152**, 215.
 Seshagiri Rao, P. & Giri, K. V. (1942). *Proc. Indian Acad. Sci. [B]*, **16**, 190.
 Sumner, J. B. (1926). *J. biol. Chem.* **69**, 435.

Blood Haemoglobin: the Relationship between Oxygen Capacity and Iron Content of Blood in Men and Women

BY Q. H. GIBSON AND D. C. HARRISON, *Department of Biochemistry, Queen's University, Belfast*

(Received 22 December 1945)

In a recent paper (Gibson & Harrison, 1945) we described the preparation of a stable artificial standard solution which matched the absorption spectrum of blood in 0.1N-NaOH over a considerable range of the visible spectrum. In order to assign a haemoglobin value to this solution for use in the estimation of blood haemoglobin, we standardized it against samples of normal male blood of known oxygen capacity and iron content. It was found that the haemoglobin values of the blood samples determined by oxygen capacity measurement were slightly, but consistently, lower than those obtained by iron estimation, a finding which agrees with

results which have been obtained by others, for example, Macfarlane & O'Brien (1944). The latter workers, however, obtained results with blood samples from women which suggested that in them this relationship is reversed, the haemoglobin value derived from oxygen being higher than that from iron. They pointed out, however, that this result was unexpected and that their investigation had not been planned to determine the extent of any such sex difference.

It seemed quite possible that if there were a systematic sex difference in the iron: oxygen ratio, there might also be a sex difference in the iron: colour

and oxygen:colour ratios, and that different haemoglobin equivalents might be required for the artificial standard with blood from the two sexes. We therefore decided to carry out two parallel series of determinations of oxygen capacity and iron content on the blood of normal men and women, taking every possible precaution to eliminate any systematic error affecting the sexes unequally.

The results would serve both to check the figures given for the standard for male blood and to establish a value for female blood if, in fact, a sex difference were found.

METHODS

A detailed description of the methods used has already been published (Gibson & Harrison, 1945), and only a brief outline will be given here. The same calibrated apparatus was used as in the above paper.

Blood samples. 30 ml. venous samples were taken from normal male and female medical students about 2-3 hr. after breakfast. A stainless steel needle and an all-glass syringe were used and coagulation was prevented by 1 mg./ml. of blood of a 2:3 mixture of potassium and ammonium oxalates. A male sample and a female sample were taken within a few minutes of one another and the two specimens were carried through all the analytical processes side by side. On alternate days the time sequence of collecting the bloods and of carrying out the subsequent processes was reversed as between the sexes, and where different apparatus was used for the two samples a similar day by day alternation was maintained.

Oxygen capacity. The determinations were carried out in duplicate with the constant-volume Van Slyke-Neill manometric apparatus. They were usually completed within 2 hr. of taking the blood samples. The values included any

carboxyhaemoglobin present. The average deviation of a determination from the mean was 0.03 g. of haemoglobin/100 ml. of blood.

Methaemoglobin. This pigment was determined photo-electrically and, where necessary, a correction was made to the observed oxygen capacity value. The figure for methaemoglobin never exceeded 0.1 g./100 ml. and was usually negligible.

Iron in blood. Iron was determined in triplicate on 3 ml. samples by the modification of the method of Klumpp (1934) previously described. The average deviation of a determination from the mean was 0.2% of the amount measured. A fresh iron standard was made from electrolytic iron (purity greater than 99.9%) and was checked with standard $K_2Cr_2O_7$. From this a dilute standard of ferric iron was prepared after oxidation with H_2O_2 . This standard was compared by $TiCl_3$ titration with a fresh dilute standard made from the strong iron standard used in our previous work. Exact agreement between the two solutions was obtained.

Measurements in the absorptiometer. The blood was diluted 1 in 101 with 0.1 N-NaOH and after mixing for a few minutes, 10 ml. of the solution were heated in a stoppered boiling tube in a boiling water-bath for exactly 4 min., a similar amount of the artificial standard being heated in the same way. After being rapidly cooled, the samples were compared in turn in the same 1 cm. glass cell against water in a Spekker absorptiometer using Hilger-Chance OGr.1 green filters. A neutral grey screen of about 0.5 density was measured immediately after each sample in order to control any chance fluctuations in the absorptiometer readings.

RESULTS

The haemoglobin values obtained from oxygen capacity and from iron content for ten men and ten women are given separately in Table 1. (The

Table 1. *Blood haemoglobin of men and women, and haemoglobin equivalents of artificial standard based on oxygen capacity and iron content*

(The figures have been calculated on the assumption that 1 g. haemoglobin combines with 1.34 ml. of oxygen and contains 0.334% Fe. The haemoglobin equivalents of the standard were derived by comparison with blood diluted 1 in 101 with 0.1 N-NaOH (Gibson & Harrison, 1945).)

	Males				Females			
	Haemoglobin in blood from		Haemoglobin equivalent of standard from		Haemoglobin in blood from		Haemoglobin equivalent of standard from	
	O ₂	Fe	O ₂	Fe	O ₂	Fe	O ₂	Fe
	(g./100 ml.)	(g./100 ml.)	(g./100 ml.)	(g./100 ml.)	(g./100 ml.)	(g./100 ml.)	(g./100 ml.)	(g./100 ml.)
	15.35	15.78	15.64	16.15	13.88	14.39	15.79	16.44
	16.67	16.94	15.88	16.27	13.62	13.78	15.84	16.13
	15.07	15.54	15.60	16.05	15.57	15.64	16.16	16.24
	16.09	16.48	16.15	16.50	16.21	16.67	15.95	16.36
	16.38	16.55	15.80	15.96	13.91	14.26	15.60	16.00
	14.76	15.09	15.68	16.06	15.55	15.85	15.68	16.01
	15.79	15.96	15.95	16.13	14.24	14.49	15.61	15.90
	16.94	17.35	15.60	16.01	15.39	15.57	15.72	15.93
	15.47	15.59	15.83	15.96	14.95	15.20	15.81	16.06
	14.65	14.96	15.59	15.95	15.06	15.41	15.82	16.18
Mean:	15.72	16.02	15.77	16.10	14.84	15.13	15.80	16.13
V%*:	—	—	1.2	1.1	—	—	1.1	1.1

* V = coefficient of variation.

figures in the second decimal place are only approximate but have been retained in order to allow the best possible estimate of the O_2 :Fe ratio.) The haemoglobin equivalent of the artificial standard based on oxygen capacity and iron determinations is given for each blood in the remaining columns.

Our results indicate that there is no difference in the O_2 :Fe ratio between men and women. The numbers examined were not large, but the average of the individual O_2 :Fe ratios was identical in each sex. The mean value corresponded to 393 ml. O_2 /g. Fe, the values ranging from 388 to 398 ml. in men and 386 to 399 ml. in women. The coefficients of variation were 0.8% in men and 0.9% in women. (The plasma Fe content is too small to affect the above results and has been neglected.)

The values for the artificial standard agree well with those derived from ten male bloods in our previous paper, the present value based on oxygen capacity being 15.78 as against 15.60 g. of haemoglobin/100 ml., and the present value from iron being 16.11 as against 16.01 found previously. The average values for the standard, based on determinations on all thirty bloods are 15.7 g. from oxygen and 16.1 g. of haemoglobin/100 ml. from Fe. We have found that there is no difference between the sexes with regard to the ratio between iron and colour and oxygen and colour, so that the same haemoglobin equivalent may be used for the standard in haemoglobin determinations carried out on the blood from persons of either sex.

To estimate the mean blood haemoglobin level of the medical students who assisted us by acting as donors in our work, the values for the ten men

who were tested in the work already published (Gibson & Harrison, 1945) were combined with those for the ten men tested on this occasion. Assuming an equivalent of 14.75 g. Hb/100 ml. for the British Standards Institution Haldane standard (Macfarlane & O'Brien, 1944; King, Gilchrist & Matheson, 1944), and using our oxygen capacity figures for the bloods, the mean Haldane value for the twenty men students was 107 (s.d. (standard deviation) 7). The blood samples were taken in 1945, and the value is almost the same as that found for fifty-two men students in 1943 in the haemoglobin survey carried out in England by the Medical Research Council Committee on Haemoglobin Surveys (1945). The average in the English survey was 106.4 (s.d. 6.4) on the British Standards Institute Haldane scale. The corresponding value for our small series of ten women medical students was 101 (s.d. 6) as compared with 92.8 (s.d. 6.8) from fifty-nine women students in the English survey.

SUMMARY

1. The ratio between oxygen capacity and Fe content is the same in the blood of men and women. The specific oxygen capacity found was 393 ml. O_2 /g. Fe (standard error of mean 0.8 ml.) compared with the stoichiometric value of 401 ml.
2. The artificial standard may be used with the same values for blood from either sex.
3. Values for the artificial standard agree closely with those previously published, the mean values from thirty bloods being 15.7 g. of haemoglobin/100 ml. of blood from oxygen capacity, and 16.1 g. from Fe.

REFERENCES

Gibson, Q. H. & Harrison, D. C. (1945). <i>Biochem. J.</i> 39 , 490.	Macfarlane, R. G. & O'Brien, J. R. P. (1944). <i>Brit. med. J.</i> 1 , 248.
King, E. J., Gilchrist, M. & Matheson, A. (1944). <i>Brit. med. J.</i> 1 , 250.	Medical Research Council Committee on Haemoglobin Surveys (1945). <i>Spec. Rep. Ser. Med. Res. Coun., Lond.</i> , no. 252.
Klumpp, T. G. (1934). <i>J. biol. Chem.</i> 107 , 213.	

Micro-method for the Determination of Vitamin A in Liver Biopsies in Man and Larger Animals

By T. K. WITH, *The Medical Department A of the Righospital, Copenhagen*

(Received 7 August 1945)

Since the liver contains over 95% of the vitamin A stored in the organism several problems concerning the metabolism of vitamin A have been elucidated by means of analyses of liver tissue—mainly in small animals, large numbers of which can be used in experiments (With, 1940, 1942). For economical

reasons experiments in which animals are killed have been few on larger animals, such as ox, horse, sheep, and swine, and many problems still remain. In man, the storage of vitamin A in the liver has not been seriously studied, although investigations might be possible by means of operation biopsies