XLIV. THE INTERPRETATION OF THE COLOUR MATCH IN THE ANTIMONY TRICHLORIDE TEST FOR VITAMIN A.

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IN a recent paper, Coward *et al.* [1931] compared the vitamin A potencies of 11 cod-liver oils and 2 concentrates, as biologically determined, with (amongst other things) the blue value of the oil determined directly and indirectly *via* the unsaponifiable matter. It was found that the blue value determined *via* the unsaponifiable matter gave better agreement with the biological determination of vitamin A than did the blue value determined directly.

It is generally observed that for oils of medicinal grade, the blue value determined *via* the unsaponifiable matter is higher than the blue value determined directly, while for very potent fish-liver oils the blue values determined directly and indirectly are in approximate agreement.

In the present paper, evidence is presented that the discrepancy between the direct and indirect determinations of the blue values of oils of medicinal grade is to be accounted for by the method of interpretation of the colour match.

The blue colours given by cod-liver oil and concentrates in the antimony trichloride reaction vary quite widely in quality. For a cod-liver oil of medicinal grade, a typical Lovibond colour match is

5.5 blue + 0.5 yellow,

while for a preparation of unsaponifiable material a typical match is

5.5 blue + 2.5 yellow – 0.8 neutral.

Exceptionally potent cod-liver oils and other liver oils give blue colours similar in quality to that given by unsaponifiable material, but various samples can be found to give colours intermediate in quality between the two examples given, and certain oils (usually old or oxidised samples) give reddish colours, such as

5.5 blue + 0.2 yellow + 1.0 red.

According to the usual interpretation the three colour matches given above represent equal amounts of chromogen, since only the value of the blue component is considered.

It seems reasonable to suppose that the preparations containing the highest concentration of chromogen give the colours nearest to what would be

given by the uncontaminated chromogen itself. That is, the colour given by a concentrate or a very potent oil is mainly due to the chromogen which it is required to estimate, and the colours given by oils of medicinal grade are different in quality because of some interfering factor or factors. The difference between the three colour matches given above is essentially a difference of redness, and it can be shown that the colour given by medicinal cod-liver oil can be reproduced by the addition of a red glass to the colour given by a concentrate. According to Heilbron, Gillam and Morton [1931] the cod-liver oil environment causes a displacement of the absorption bands of the chromogen itself towards the blue end of the spectrum. Thus the colour given by cod-liver oil is more reddish than that given by a concentrate.

Experiments with copper sulphate solution.

To investigate the effect of varying colour quality on the blue component in the colour match, some experiments have been made with a copper sulphate solution observed through Lovibond red glasses of various values. The actual colour matches (in each case the average of the readings made by two or three observers) are given in Table I.

Table I.

Copper sulphate solution covered by a red glass of value	Colour match				Blue minus yellow
	Blue	Yellow	Red	Neutral	
6.0	3.83	0.23	2.13	—	3.60
5.0	3.85	0.25	1.1	—	3.60
4.0	4.06	0.36	0.53	—	3.70
3.0	4.2	0.5	0.1	—	3.7
2.0	4.5	0.9	—	—	3.6
1.0	5.45	1.75	—	-0.55	3.70
0.0	6.9	3.33	—	-1.13	3.57

These colour matches cover a range wider than that usually found in cod-liver oils and concentrates, but matches such as those given by the copper sulphate solution covered by Lovibond red glasses of 3.0, 2.0 and 1.0 are frequently encountered. It is observed that the value of the blue component increases throughout, and very rapidly in the last three matches.

This variation of the blue value with the redness of the combination of copper sulphate solution and red glasses is not surprising, for, since the neutral glass consists of red, yellow and blue glasses of equal value bound together, the effect of the progressive removal of red on the first colour match of Table I can be calculated (on the assumption that the glasses are additive) as follows.

Table II.

(3.83 blue + 0.23 yellow + 2.13 red)	- 1 red =	3.83b + 0.23y + 1.13r.
" "	- 2 red =	3.83b + 0.23y + 0.13r.
" "	- 3 red =	4.7b + 1.1y - 0.87 neut.
" "	- 4 red =	5.7b + 2.1y - 1.87 neut.
" "	- 5 red =	6.7b + 3.1y - 2.87 neut.
" "	- 6 red =	7.7b + 4.1y - 3.87 neut.

When there is no red glass in the colour match, the effect of removing red is to add blue + yellow – neutral in equal proportions. By comparing Table II with Table I it is seen that the value of the blue component would be expected to increase even more rapidly than is actually found.

From Table I it is evident that the value of the blue component could not be taken as an index of the amount of copper present in a solution whose colour quality varied in the same way as the blue solutions given by cod-liver oils and concentrates vary. The amount of copper in the solution used in the above matches remained constant, but the value of the blue component did not, and as shown in Table II, it could not be expected to. The only simple expression derived from the colour match which could be expected to remain constant is the value “blue *minus* yellow,” and reference to Table I shows that this is found to remain practically constant independent of the redness of the combination of copper sulphate solution and red glass. The highest value found was 3.7 and the lowest 3.57, a difference well within the limits of error in carrying out the matches.

“Blue minus yellow” values of cod-liver oils and concentrates.

From the above considerations it would be expected that the value “blue *minus* yellow” would furnish a better measure of the chromogen of cod-liver oil than the blue value alone.

An experiment was made in which ergosterol, which gives a red colour with antimony trichloride solution, was added to a vitamin A concentrate. The match of a 0.25 % solution of concentrate was

7.5 blue + 3.0 yellow – 0.8 neutral.

A 0.1 % solution of ergosterol gave with the antimony trichloride reagent a red colour increasing in intensity. The match at about 20 seconds was

2.0 red.

The match of the two solutions in superimposed cells, or in a solution containing together the same amounts of the concentrate and ergosterol as were used in the separate matches, was

5.0 blue + 0.7 yellow.

The addition of the red chromogen lowered the blue value of the concentrate in the ratio 7.5/5, but the value for “blue *minus* yellow” remained practically unchanged.

Just as the blue value of a concentrate is lowered by the addition of a red chromogen, so the blue value of a cod-liver oil is increased by the removal of redness on rejection of the saponifiable material. The blue value of an oil was determined directly by the method recommended by the General Medical

Council [1931] and indirectly *via* the unsaponifiable matter. The results expressed in full were:

Colour match of original oil calculated to 20 % wt./vol. solution:

11.8 blue + 1.25 yellow.

Blue value = 11.8.

"Blue *minus* yellow" value = 10.55.

Colour match on unsaponifiable matter calculated to 20 % wt./vol. solution of original oil:

14.7 blue + 4.25 yellow - 1.18 neutral.

Blue value = 14.7.

"Blue *minus* yellow" value = 10.45.

The decrease of redness on saponification and extraction causes a rise in the blue value in the same way as the removal of red from the combination of copper sulphate and red glasses causes a rise in the blue component of the colour match, and in both cases the value for "blue *minus* yellow" remains practically unchanged.

I have been able to examine oil A and oil L in the series assayed by Coward *et al.* [1931]. The colour matches determined were:

Oil A. 0.2 cc. of a 10 % solution:

5.5 blue + 0.4 yellow.

Blue value = 11.0.

"Blue *minus* yellow" value = 10.2.

Oil L. 0.2 cc. of a 0.35 % solution:

5.5 blue + 2.5 yellow - 0.8 neutral.

Blue value = 314.

"Blue *minus* yellow" value = 171.

The ratio of the blue values of these oils is oil L/oil A = 28.5/1, and the ratio of the "blue *minus* yellow" values is 16.8/1. The ratio of their biological values is given in Coward's paper as 15.6/1.

SUMMARY.

It is known that the vitamin A potencies of very potent oils and concentrates compared with a medicinal oil as standard are lower than would be expected from their blue values. It is also known that the blue value of a medicinal grade oil determined *via* the unsaponifiable fraction is higher than the blue value determined directly. These discrepancies are attributed to the influence of varying colour quality on the blue component of the colour match. It is shown that the blue reading in the Lovibond match of a copper sulphate solution varies widely as the quality of the light from the solution is varied by the interposition of red glasses. The variation obtained is similar to that given in the colour matches on a wide range of oils and concentrates.

It is also shown that the value for "blue *minus* yellow" can be expected to remain constant however the redness of the solution varies, and it is considered that the value "blue *minus* yellow" would be a better criterion of vitamin A than the blue value as usually read. Biological tests and the fact that the "blue *minus* yellow" value of an oil determined *via* the unsaponifiable fraction agrees with the value determined directly support this conclusion.

REFERENCES.

- Coward, Dyer, Morton and Gaddum (1931). *Biochem. J.* **25**, 1102.
 General Medical Council (1931). Pharmacopoeia Commission. Report of Cod-Liver Oil Colour Test Sub-Committee. March.
 Heilbron, Gillam and Morton (1931). *Biochem. J.* **25**, 1352.