Colour intensities of Feulgen-stained nuclei expressed in relative values

Type of measurement	Type of tissue and cell	Type of rinsing solution		Degree of colour augmentation caused by rinsing with B $\frac{B}{A}$
		A	B	
Microspectrophotometry[1]	liver; nuclei of group II assumed as tetraploid[3]	8·04 ± 0·20[4] (n = 25)	11·36 ± 0·18[4] (n = 26)	1·41 ± 0·04[5]
Microspectrophotometry	testis; first spermatocyte, leptotene stage	9·68 ± 0·10 (n = 30)	12·22 ± 0·25 (n = 29)	1·26 ± 0·03
Microspectrophotometry	kidney; cells of convoluted tubules	4·57 ± 0·07 (n = 26)	6·30 ± 0·22 (n = 26)	1·38 ± 0·05
Chemical determination[2]	liver; isolated nuclei.	0·113 ± 0·001 (n = 2)	0·152 ± 0·000 (n = 2)	1·34 ± 0·01

[1] Data are expressed in r^2E which should be proportional to the total amount of the Feulgen pigment present in the nucleus measured, r and E being radius and extinction at 5461 Å of the nucleus, respectively.

[2] Data are expressed in E_f/E_d where E_f is extinction due to fuchsin and E_d that due to hydrolyzed D.N.A. of hot perchloric acid extract of the stained nuclei, both being measured at respective absorption maxima.

[3] Values of r^2E for liver nuclei must not be compared directly with those for testis and kidney.

[4] Mean and standard error of mean, n being number of the nuclei measured through microspectrophotometry or number of parallel runs in chemical determination.

[5] Standard errors were calculated from means and standard errors of A and B.

Using the analytical methods employed here, experiments are now in progress for exploring quantitative aspects of the Feulgen reaction *in situ*.

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Zusammenfassung

Auswaschen der Feulgen-gefärbten Präparate mit auf ein pH von 2,28 gepufferter, 1,5%iger Lösung von $Na_2S_2O_5$ an Stelle der allgemein gebräuchlichen Spülflüssigkeit ergibt eine intensivere Kernfärbung. Die beobachtete Zunahme der Farbstärke ist mikrospektrophotometrisch sowie chemisch quantitativ bestimmt worden. Mit beiden Methoden hat sich eine grundsätzliche Übereinstimmung der Werte für verschiedene Zellarten erzielen lassen.

Spectrophotometric Characteristics of "Regenerated" Blood *in vitro* after Saturation with CO

Several authors, such as Haxthausen[1], Krötz[2], Fröhlich, and Rodenacher[3], Schilling Siengalewicz, and Puchowski[4], using photographs methods, have proved that the oxygenated blood appears opaque to the radiations of the first infra-red, while the carbon-monoxide blood shows an almost perfect transparency in this spectral zone.

Merkelbach[5], in 1935, controlled these results, using haemoglobin obtained from washed red blood cells and then haemolysed by a cold treatment, and he proved that the behaviour noted by previous authors was due to an absorption band present in HbO_2 between 700 and 1300 mμ. This band is completely absent in HbCO. He pointed out the importance of this for possible applications to clinical investigation.

In 1938, we studied these applications, suggesting an easy technique of HbCO dosage by infra-red photography[1].

However, Eggert[2] in 1935, noted, by photographic and spectrographic means, that blood saturated with CO and subsequently "regenerated" with a draft (that is, the blood in which the CO combined with the haemoglobin is again substituted by O_2), showed in the first infra-red, between 700 and 1000 mμ, a transparency almost equal to that of the blood saturated with CO and not "regenerated".

In 1938, Truffert[3] obtained similar results, not only with "regenerated" blood *in vitro*, but even with "regenerated" blood *in vivo*, through spontaneous or therapeutical elimination of CO on surviving patients after an acute poisoning or on patients who were recovering from a chronic poisoning.

This spectral characteristic of the "regenerated" blood, very analogous to that of poisoned blood and very different from that of normally oxygenated blood, might reopen the whole discussion of CO poisoning pathogenesis, both acute and chronic, and it might also explain many points which are as yet unknown.

For that reason we have thought it advisable first of all to control the results obtained *in vitro* by Eggert[4] and Truffert[5].

Technique: The following samples of the same human blood, fluoridised and haemolysed with saponine, have been tested by spectrophotometric methods:

(1) blood oxygenated through prolonged agitation in the presence of air;
(2) carbon-monoxide blood made by prolonged agitation in CO;

[1] N. Haxthausen, Derm. Wschr. *35*, 1219 (1933).
[2] A. Krötz, Med. Ges. in Hamburg (1934).
[3] A. Fröhlich and G. Rodenacher, Münch. med. Wschr. *4*, 146 (1935).
[4] S. Schilling-Siengalewicz and B. Puchowski, Zacchia [2] *1*, No. 1 (1927).
[5] O. Merkelbach, Schweiz. med. Wschr. *65*, 1142 (1935).

[1] V. Perelli, Diagn. Tecn. Labor. *9*, No. 6, 407 (1938).
[2] J. Eggert, Agfa *4*, 110 (1935).
[3] L. Truffert, Bull. Soc. méd. Hôp. Paris *55*, 745 (1939).
[4] J. Eggert, Agfa *4*, 110 (1935).
[5] L. Truffert, Bull. Soc. méd. Hôp. Par. *55*, 745 (1939); Arch. Mal. Prof. *5*, 78 (1943).

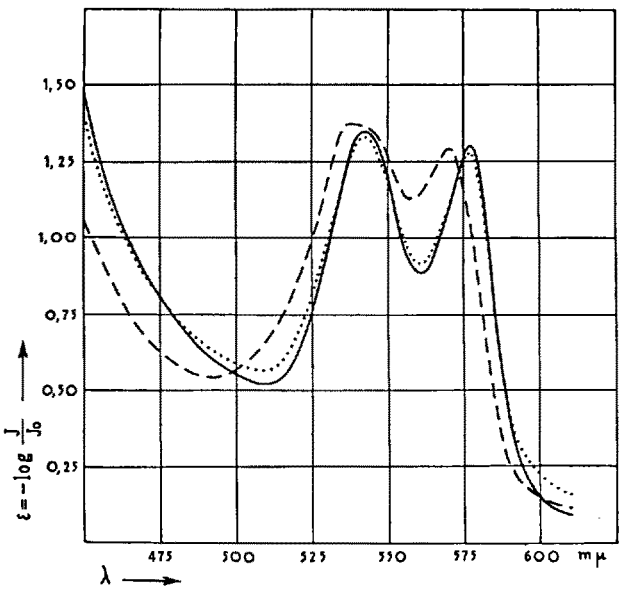

Fig. 1. 1% solution.

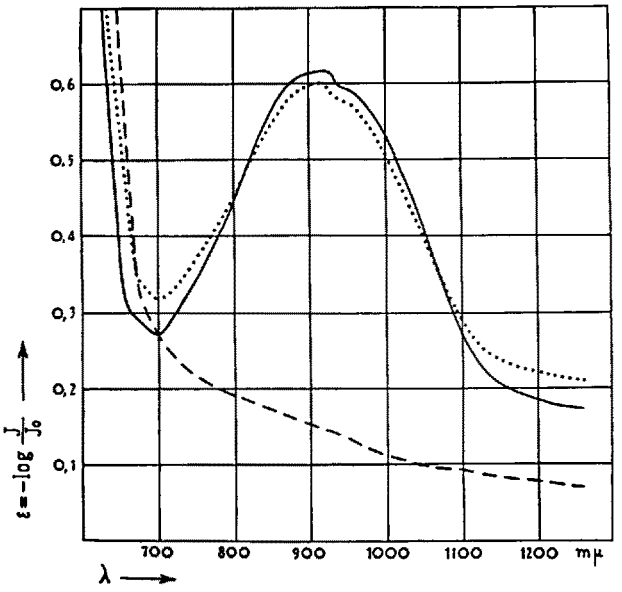

Fig. 2. 20% solution.

Thickness 10 mm; unbroken line: oxygenated blood; line with dashes: carbon-monoxide blood; dotted line: "regenerated" blood.

(3) "regenerated" blood. (The regeneration has been obtained by submitting the carbon-monoxide blood to half-an-hour's draft and irradiating it simultaneously by ultraviolet rays.)

The degree of oxygenation, carbon-monoxidation and regeneration of the three samples of blood was controlled by the extraction and dosage of the gases contained, following NICLOUX's[1] technique, modified by KOHN-ABREST[2].

The spectral field of vision (from 450 to 600 mμ) was investigated with a 10 mm thickness and 1% dilution, while a 20% dilution with the same thickness was used for the spectral field of the first infra-red (from 600 to 1200 mμ). The Mod. D.U. Beckman's spectrophotometer was used[3].

Results obtained: Among results of numerous tests made with human blood, which were all substantially in agreement, we present the ones obtained by a test which, in our opinion, gave particularly demonstrative results.

Haemoglobin gr. 14·92%.
Present gases in oxygenated blood:
CO_2 34%, O_2 23%, CO 0·10%.
Present gases in carbon-monoxide blood:
CO_2 16%, O_2 non-dosable traces, CO 22%.
Present gases in "regenerated" blood:
CO_2 non-dosable traces, O_2 22%, CO 0·60%.

As shown on Figures 1 and 2, we have drawn the diagrams for visible and first infra-red by putting wave lengths on abscissae and the extinction coefficients ($\varepsilon = -\log J/J_0$) obtained on ordinates.

In the visible field, the values obtained for oxygenated blood and for carbon-monoxide blood tally with the known ones, while it is evident that the behaviour of the "regenerated" blood is very near to that of carbon-monoxide blood.

In the first infra-red field the results obtained by MERKELBACH are confirmed for oxyhaemoglobin and carbon-monoxide haemoglobin, with the presence of a large absorption band for the former. However the behaviour of the "regenerated" blood in this spectral field does not confirm the results obtained by EGGERT and TRUFFERT, as it does not show the permeability noted by these authors, but an absorption band which is identified with the one shown by the oxygenated blood.

Conclusions: These results lead us to the conclusion that the "regenerated" blood *in vitro*, after saturation with CO, shows some spectrophotometric characteristics which allow it to be identified with normal oxygenated blood, at least with regard to the chromatic component. But oxygen transport function exercised by haemoglobin *in vivo* being attached just to this component, it must be supposed that "regenerated" blood may completely reacquire this function in the organism, without having been altered by the short combination with CO.

This hypothesis has its confirmation in the clinical experience of the quick disappearance of symptoms of acute poisoning, which is generally verified, simultaneously to the ready disappearance of CO from the blood.

Our results do not allow us to put forward a hypothesis to explain the aspects of CO poisoning pathogenesis which are still unknown, but they permit us to state, in the contrary to what has been maintained by other authors, that there is a close parallelism between the CO quantity present in the blood and its spectral characteristics.

V. PERELLI

Department of Legal Medicine, University of Genoa, January 30, 1952.

Riassunto

L'autore ha studiato, in base alle caratteristiche spettrofotometriche nel visibile e nel primo infrarosso, il sangue ossigenato, il sangue carbossidato e quello rigenerato dal precedente con una corrente d'aria, dimostrando che il sangue rigenerato *in vitro* presenta caratteri spettrali analoghi a quelli del sangue ossigenato, contrariamente a quanto avevano sostenuto precedenti autori.

[1] M. NICLOUX, *L'Oxyde de Carbone et l'Intoxication Oxycarbonique* (Masson, Paris, 1925).

[2] E. KOHN-ABREST, *Précis de Toxicologie* (Doin, Paris, 1948).

[3] For technical details refer to: V. PERELLI, Atti 11° Congr. naz. Med. legale Assicur.; Catania-Taormina, 30 maggio a 2 giugno 1951; Minerva Medico-legale *70*, 147 (1950); *71*, 26 (1951).