thymus. La vérification expérimentale de cette assertion a montré en effet que l'action de 100 γ dans ces conditions n'est pas mesurable à l'aide de notre épreuve. Dans le lot des cobayes qui ont reçu 100 γ, la moitié environ a réagi aux injections simultanées de thyroxine et de thymus comme avant l'irradiation (10 unités-cobaye annulent 10 γ de thyroxine); chez les autres, la dose suffisante de thymus augmente dans une mesure très différente d'un animal à l'autre. Il en résulte une dispersion telle des résultats individuels qu'une mesure satisfaisante est impossible. Ceci nous semble justifier l'assertion que la dose de 100 γ est très proche du seuil d'action des rayons X dans notre épreuve.

Entre 200 et 400 γ, les résultats calculés à l'aide de la formule proposée plus haut se sont rangés à 104,5 $\pm$ 10,7 % des résultats expérimentaux.

Nous tenons à remercier une fois de plus le Dr CH. M. GROS, professeur agrégé, qui dirige le centre régional anticancéreux de Strasbourg et qui nous a accordé son hospitalité pour ce travail.

J. COMSA

Clinique infantile de l'Université de Strasbourg et Centre régional anticancéreux de Strasbourg, le 16 avril 1952.

Summary

(1) The classical knowledge of the morphological changes determined by the X-rays in the thymus permitted the supposition that X-ray treatment might result in a decreased efficiency of the thymus hormone.

(2) An attempt was made to prove this assumption by measuring the antithyroid potency of the thymus extract of BEZSSONOFF and COMSA.

(3) These measurements have shown that the antithyroid potency of this extract decreased notably in X-ray-treated guinea pigs. This effect of the X-rays decreases in a second-degree function of the time elapsing after irradiation and becomes non-measurable within about 20 days.

Enhancement of Colour Intensity in the Histochemical Feulgen Reaction: Method and Quantitative Estimation

The colour intensity of the Feulgen reaction performed in test-tube with a solution of D.N.A. is much dependent on the acidity and the concentration of sulfurous acid of the medium[1]. Thus, optimal pH for the colour development is around pH 2·3[2], and addition of acids to the coloured solution accelerates the decomposition of pigment once formed[3]; increased concentration of metabisulfite further enhances the development of the Feulgen colour[4].

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Meanwhile, in the Feulgen reaction conducted *in situ*, excess of the Schiff reagent or leucofuchsin must be removed from the coloured tissue preparations without causing regeneration of the native fuchsin dyestuff which otherwise may give rise to an extraneous staining. FEULGEN and ROSSENBECK[1], the originators of the histochemical Feulgen reaction, employed for this purpose a rinsing solution prepared by adding 10 ml of 10 % sodium bisulfite (or metabisulfite) and 10 ml of NHCl to 200 ml water, and this has invariably been followed by subsequent workers essentially without modification[2] except HEITZ[3] who has used 45 % acetic acid for this purpose. However, a rinsing solution of such a composition is remarkably acidic (about 0·05 NHCl) and its sulfurous acid content is suboptimal for the colour development. Accordingly, it is reasonably suspected that a more intense coloration of the Feulgen stain might be realized if a solution for an optimal Feulgen colour-reaction were used for rinsing.

We tested this possibility by comparing colour intensities of Feulgen-stained preparations which had been rinsed with either of the two solutions: (*A*) the rinsing solution now commonly in use[2] (for the composition, see above); (*B*) a mixture of 9 volumes Sörensen's 0·1 N glycine buffer of pH 2·28 and 1 volume 15 % sodium metabisulfite. The composition of the latter solution is similar to that of the medium established by SIBATANI[4] for the Feulgen reaction *in vitro*. From this experiment it appeared likely by a visual inspection that rinsing with solution *B* effected more intense coloration than solution *A*. Repeated experiments gave consistent results.

Then, the amount of the Feulgen pigment produced in cell nuclei was estimated with several rat tissues fixed with 50 % formalin. This was done in order to obtain decisive evidence for the augmentation of the colour intensity, and also to answer the question whether proportions of the colour enhancements with solution *B* are of the same order for cell nuclei of different types. This was done through microspectrophotometric measurements using the apparatus of the Koana-Naora system[5]. As shown in the accompanying table, the values indicating the amounts of the pigment produced in the individual nuclei are decidedly higher with solution *B* for three cell types tested. Moreover, the degree of the colour augmentation is fairly constant among these cells, which are distinct from each other in the total amount of the pigment produced within a single nucleus under given conditions of rinsing.

Recently, we have worked out a method which allows to estimate chemically the relative concentration of fuchsin to D.N.A. in Feulgen-stained nuclei[6]. With rat liver nuclei isolated in citric acid and fixed with 20 % formalin, determinations were made for each of the two rinsing solutions indicated above. It should be noted from the data presented in the table that the degree of the increment in the colour intensity as estimated by the chemical method falls within the range of variation of the corresponding values obtained with nuclei of different cell types through microspectrophotometry.

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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;

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