HISTOCHEMISTRY

Cytophotometric Determinations of Basic Proteins of Cell Nuclei with Basic Dyes

Until now only alkaline fast-green staining[1] has been used to any extent for the histochemical detection of basic nuclear proteins. For the determination of DNA, the Feulgen reaction as well as gallocyanin chromalum (GC) staining have proved successful[2]. The amino and guanido groups of proteins react stoichiometrically with metaphosphoric acid[3,4]. After pretreating cells with metaphosphoric acid it is possible to bind a basic dyestuff like GC indirectly to basic groups of proteins. By comparing biochemical and histochemical data, it can be shown that fast-green staining at *p*H 8·2 as well as the GC-metaphosphate technique give closely similar results for histones.

Bull sperm, calf thymus lymphocytes, trout erythrocytes and chicken erythrocytes were fixed in 96 per cent ethanol for 1 h. Fast-green staining was done according to Alfert and Geschwind[1]. For GC-metaphosphate staining, slides were treated with 5 per cent TCA at 90° C for 15 min, then incubated in 0·1 M metaphosphoric acid (Merck, Darmstadt) at 20° C for 1 h, followed by gallocyanin chromalum according to Sandritter *et al.*[2]. The Barr and Stroud integrating microdensitometer was used for the cytophotometric measurements (570 mμ for the Feulgen reaction, 600 mμ for fast-green staining, 500 mμ for gallocyanin chromalum staining).

With the alkaline fast-green staining for determining basic proteins, a relationship is found between the measured values of bull sperms, calf thymus lymphocytes, trout erythrocytes and chicken erythrocytes as 1 : 1·9 : 1·4 : 0·75. There is a similar ratio with the metaphosphate-gallocyanin chromalum technique (1 : 1·9 : 0·8 : 0·6). The trout erythrocytes showed the only deviation (decrease of fast-green staining). The cause of this deviation is not quite clear.

After deaminating the NH_2 groups of proteins with HNO_2 (5 per cent at 20 min, 20°) one may expect to obtain a selective staining of the guanido groups of arginine. As shown in Table 1, both staining reactions (fast-green staining *p*H 8·2 and metaphosphate-gallocyanin chromalum staining) show identical results after deamination, only the ratios differing slightly. The values correspond very well with the arginine values obtained by biochemical determinations[5,6] (see Table 1, columns 2, 4). In the same way the DNA/arginine proportion (Feulgen/arginine—see Table 1, columns 3, 5 and 6) is the same for both staining reactions and also corresponds with the biochemical data.

These measurements demonstrate that with histochemical techniques in connexion with cytophotometry, it is possible to make semi-quantitative determinations of basic nuclear proteins. Two staining methods involving totally different techniques produce similar results and are in agreement with the biochemical data.

The metaphosphoric acid technique, as outlined here, may also be used with other basic dyes (that is, toluidine blue) including fluorescent dyes (that is, acridine orange, rivanole).

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PATHOLOGY

Modification of X-ray Survival Curves of Stem-cells by Different Doses of Erythropoietin

A previous study of stem-cell response to erythropoietin in the polycythaemic mouse after 150 rads showed an initial depression to values between 5 and 15 per cent of unirradiated controls[1]. Gurney[2], however, in an earlier publication, reported an erythropoietin response after a single dose of 150 rads of about 30–40 per cent of control. The experiments were similar except for the dose of erythropoietin used: in our experiments about 0·5 unit (M.R.C. Standard '*A*')[3] and in Gurney's 6 units Armour Company erythropoietin (≈10 units M.R.C. Standard '*A*'). We therefore decided to investigate whether or not the dose of erythropoietin influences the apparent radiation effect in this system.

In the present study two different doses—0·6 unit Armour Company erythropoietin (≈1 unit M.R.C. Standard '*B*') or a 10-fold concentration (≈10 units M.R.C. Standard '*B*')—were used. Polycythaemia was created by exposing $(C_3H \times AKR)F_1$ hybrid mice to half an atmosphere of air for 10 days (ref. 4), and then giving a single intraperitoneal injection of 0·8 ml. of washed red cells suspended in 0·2 ml. saline. On the 5th day after the transfusion mice were irradiated in groups of eight (four from each erythropoietin dose group) with different whole-body doses of X-rays (300 kVp., 'Resomax', half value thickness of 1·9 mm Cu at a source to surface distance of 70 cm and a measured dose-rate of 60 rads/min). Erythropoietin was injected intraperitoneally in 0·5 ml. saline immediately after irradiation and animals were afterwards housed in colony cages. Fifty-two hours after erythropoietin, 1·5 μc. ^{59}ferric citrate (specific activity

Table 1

Col.	Pretreatment and staining	Object*			
		Bull sperm AU	Calf thymus AU	Trout erythr. AU	Chicken erythr. AU
1	Feulgen reaction (DNA)	18 ± 1·1 (n = 100)	37 ± 1·6 (n = 150)	28 ± 1·4 (n = 100)	16 ± 0·79 (n = 100)
2	TCA + deamination + metaphosphoric acid + gallocyanin chromalum (arginine)	11 ± 1·6 (n = 150)	10 ± 2·1 (n = 200)	6 ± 1·6 (n = 100)	5 ± 0·72 (n = 100)
3	Ratio $\frac{\text{DNA}}{\text{Arginine GC}}$	1·7	3·7	4·7	3·4
	Proportion	1	2·6	2·9	2·3
4	TCA + deamination + fast green *p*H 8·2 (arginine)	7 ± 0·8 (n = 150)	5 ± 1·1 (n = 200)	3 ± 1·5 (n = 100)	2 ± 1·5 (n = 100)
5	Ratio $\frac{\text{DNA}}{\text{Arginine FG}}$	2·6	7·4	9·0	8·0
	Proportion	1	2·8	3·5	3·1
6	Ratio $\frac{\text{DNA}}{\text{Arginine}}$ (ref. 6)	1·5	4·3	4·9	4·4
	Proportion	1	2·9	3·3	2·9

* Data in columns 1, 2 and 4 expressed as mean dye content per nucleus and standard deviation.