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Preliminary Communications

Deoxyribonucleic Acid Content of Epidermal In-situ Carcinomas

Previous observations indicated that most carcinomas and sarcomas of man were composed of cells characterized by abnormal deoxyribonucleic acid (D.N.A.) values (Bader, 1959; Richards and Atkin, 1959; Stich *et al.*, 1960; Stich and Steele, 1962), whereas cells of several benign tumours had a normal diploid D.N.A. content (Stich *et al.*, 1960; Stich, 1962). Apart from these two types of neoplasm there is an epithelial proliferative lesion known as in-situ carcinoma, the true nature and eventual development of which is still ill-defined. It appeared, therefore, of interest to study the D.N.A. content in the neoplastic cells of these lesions in man, and to determine whether their D.N.A. content resembles that of benign or malignant cells.

The surgically excised lesions were fixed in 10% neutral formalin, embedded in paraffin, sectioned at 8 μ , and stained with Feulgen's technique. Patau's (1952) two wavelength method with Mendelsohn's (1958) calculation tables was used to estimate the relative amount of D.N.A. About 75 metaphase plates and 30 lymphocytes were measured per sample. The D.N.A. content of lymphocytes was used to give the diploid D.N.A. value of a normal cell. The symbol C denotes the amount of D.N.A. corresponding to a nucleus with haploid chromosome number. Thus a nucleus belonging to a diploid class has a D.N.A. amount of 2C in telophase nuclei and 4C in metaphase plates (Stich and Steele, 1962). The incidence of mitotic irregularities was estimated on Feulgen stained sections.

Metaphase plates of normal epidermis and of in-situ carcinomas were used for the D.N.A. measurements because at this particular stage of the mitotic cycle no D.N.A.

synthesis occurs. Thus deviations in chromosome number from a diploid complement will be reflected in either an increased or a decreased D.N.A. content. All normal epidermal cells examined showed D.N.A. values which fell into the expected D.N.A. class for diploid cells (see Fig.). In contrast, the cells of in-situ carcinomas showed a great variability in the D.N.A. values. In eight cases they extended from the diploid to a hypertetraploid range (Cases 418, 2446, 6496, 5552, 57, 1859, 495, and 4778), in three cases (1407, 5669, and 3990) to hyperoctoploid range, and in one case (2224) to a sub-sixteen ploid range. These findings show that the lesions examined consist of heterogeneous cell populations in regard to their D.N.A. values. A dominant cell type characterized by a particular aneuploid D.N.A. value was not observed. In this regard, the lesion examined seems to differ from most invasive carcinomas, which, while showing a heterogeneous composition, have usually a particular stemline (Bader, 1959; Stich, 1960; Stich *et al.*, 1960; Stich and Steele, 1962).

Another consistent finding in the cases of in-situ carcinoma examined was a relatively high incidence of mitotic irregularities, including chromosome bridges, fragments, and multipolar mitosis (see Table). It is very likely

Incidence of Mitotic Irregularities in Normal Skin and Carcinomas In-situ

Specimens	Case No.	No. of Cells Counted	Mitotic Irregularities (%)			
			Bridges	Frag-ments	Multi-polar Mitosis	Total
Normal skin	236-55	250				2.1
	2092-55	265				3.2
	2133-55	260				1.9
	2224-56	255				2.6
	5552-58	275				1.4
	4940-62	66				3.0
In-situ carcinomas	3990-56	85	14.4	5.8	1.1	21.3
	2224-57	91	23.0	3.2	0.0	26.2
	495-57	109	22.0	1.8	0.9	24.7
	4778-58	66	19.6	7.5	0.0	27.1
	418-58	84	13.0	4.7	1.1	18.8
	1859-58	65	6.1	4.6	0.0	10.7
	5552-59	110	16.3	3.6	0.0	19.9
	1407-61	108	34.2	2.7	0.0	36.9
	5669-62	72	16.6	2.7	1.3	20.6
	2446-62	93	16.1	6.4	0.0	22.5
	6496-62	84	15.4	5.9	1.2	22.5
	57-63	80	10.0	7.5	2.5	20.0

that these mitotic abnormalities led to the formation of the observed variation in D.N.A. values.

The occurrence of a genetically heterogeneous population and the absence of a dominant aneuploid stemline in the in-situ carcinomas resembles the pattern found in pre-neoplastic livers of rats fed various liver carcinogens (Stich, 1960, 1962) and to the pre-leukaemic lesions in mice injected with 7,12-dimethylbenz(α)anthracene (D.M.B.A.) (Joneja and Stich, 1963). The hepatomas and thymic lymphosarcomas consisting of cells with abnormal chromosome complement originated from this genetically heterogeneous population. It seems possible, therefore, that the heterogeneous cell population of the in-situ

Distribution of D.N.A. values in normal skin and in-situ carcinomas.

carcinomas provides the opportunity for the selection of a dominant cell type, which characterizes the invasive growth.

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Medical Memoranda

Blood Groups and Enzymes of Human Red Cells After Two Years' Storage in Liquid Nitrogen

The possible advantages of liquid nitrogen for preserving red cells for grouping purposes were reported in a previous paper (Huntsman, Hurn, Ikin, Lehmann, and Liddell, 1962). The present communication describes the results found after the storage period had been extended from one to two years. The methods employed were identical to those previously reported.

RESULTS

1. *Recovery of Intact Cells.*—The average recovery in eight samples was 92.8% (range 90 to 96%), compared with the average recovery after one year of 94.1% (range 93 to 96%). It was noted with all specimens that haemolysis in the thawed cell suspensions continued during the next twelve hours while the specimens were held at 4° C. This feature was more pronounced with the two-year specimens.

2. *Grouping Properties.*—Preliminary "run-through of all groups" of the same seven samples showed no deterioration when thawed after two years' storage. Titrations of all the antigens tested previously showed no significant alterations.

3. *Enzyme Estimations.*—The glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities were well maintained, and there was in fact some increase of activity, as was also seen after one year. This could be due to the release of an enzyme activator in the stroma. The deterioration in the glutamic-oxaloacetic transaminase and the aldolase continued, dropping to 80% and 50% of the original levels respectively (see Chart). This is the typical enzyme pattern seen on storage at 4° C., as distinct from *in vivo* ageing of the red cells (Rapoport, 1961). Its progress during the present experiment confirms our observation after the first year of storage, that complete

stability of biological systems has not been obtained even at the very low temperature of -196° C.

COMMENT

The recovery of red cells stored for two years in liquid nitrogen averages 92%. The grouping properties of these cells are satisfactory, but the thawed cell suspensions show an increased tendency to progressive haemolysis. If this lysis after thawing increases with more prolonged storage, this may prove to be the factor which ultimately limits the effective storage life. The red-cell enzymes showed no fall in original activity of two dehydrogenases, but there was further deterioration in the second year for glutamic-oxaloacetic transaminase and for aldolase.

Enzyme activity of red cells during two years' storage in liquid nitrogen. Results expressed as a percentage of the level before freezing. (1) Aldolase. (2) Glutamic-oxalacetic transaminase. (3) Glucose-6-phosphate dehydrogenase. (4) 6-Phosphogluconate dehydrogenase.

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