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The Histoenzymological Pattern of Human Breast Carcinoma

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Abstract—*The histoenzymological pattern of a number of enzymes was investigated and correlated to certain clinicopathological parameters in 50 cases of human breast carcinomas. The examined dehydrogenases and diaphorases show either an increased or equal activity to the adjacent normal epithelium. The activity of phosphatases appears increased in cancer cells with the exception of 5-nucleotidase, while the observed activity of β-glucuronidase, MAO, NSE and LAP appears variable. It was found that a number of dehydrogenases as well as MAO and NSE have an activity related to the grade of malignancy of the tumour. It was found as well that SDH, G6PDH and MAO activities are related to the hormonal status. Finally cytochrome oxidase was found increased in cancer cells in cases with secondaries in the axillary lymph nodes.*

INTRODUCTION

HISTOENZYMOLOGICAL studies in human tumours revealed a possible clinical significance of the enzyme histochemical differences between individual carcinomas of an organ [1]. In human breast carcinoma this significance will be more reliable if there is a relationship between the histoenzymological pattern and pathological parameters which are known to be important for the prognosis and biological behavior of the tumour. The purpose of this paper is the investigation of the pattern of a number of enzymes in relation to certain of the previous parameters.

MATERIALS AND METHODS

Tissue blocks from cases of breast carcinomas, obtained from radical mastectomies within 4–6 min from excision, were snap-frozen in liquid hexane at −70°C using solid CO_2 and absolute alcohol mixture as coolant. Serial cryostat sections were cut at 6–8 μm. Two sections, a test and a control, were mounted directly onto each slide, to be used for the study of the enzymes. Several other sections were fixed in Wolman's acetic acid–alcohol fixative [2] for 10 min and stained with haematoxylin and eosin.

Corresponding samples of tissue and the axillary lymph nodes were proccssed by the parafin method for routine histology.

From the initial number of cases 50 were selected which allowed a comparison of the tumour tissue to the normal adjacent epithelium. The sections were incubated in the appropriate media using the open perspex ring technique [3]. The examined enzymes are presented in Table 1.

The grading of enzyme activity was done subjectively by viewing with a low power objective and characterised as increased, equal or decreased in comparison to the activity of the adjacent epithelium. The following parameters were considered for the estimation of the results: age, presence of menstrual cycle, gross diameter of the tumour, grade of malignancy [4] and the presence of secondaries in the axillary lymph nodes.

Statistical analysis was done by using χ^2 test.

RESULTS

Histology of the tumour, grade of malignancy, presence of menstruation and presence of secondaries are presented in Table 2.

The examined dehydrogenases and diaphorases show either an increased (the majority of cases) or an equal activity to the

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Table 1. The examined enzymes and the used substrates and incubation periods

Enzyme	Substrate	Incubation (min)	Temp. (°C)
Succinate DH	Sodium succinate	30	37
Lactate DH	Sodium lactate	30	37
Glutamate DH	Sodium lactate monohydrate	30	37
Glucose-6-phosphate DH	Glucose-6-phosphate	30	37
Alcohol DH	Ethanol	30	37
Isocitrate DH	L-Isocitrate	30	37
Malate DH	L-Malate	30	37
Hydroxybutyrate DH	DL-b-Hydroxybutyrate	30	37
NADH diaphorase	NADH	30	37
NADPH diaphorase	NADPH	30	37
Acid phosphatase	Naphthol AS-BI phosphate	30	37
Alkaline phosphatase	Naphthol AS-BI phosphate	30	37
Adenosine triphosphatase	ATP disodium salt	60	37
Glucose-6-phosphatase	Glucose-6-phosphate	30	37
5-Nucleotidase	Adenosine-5-monophosphate	60	37
Leucine aminopeptidase	L-Leucyl-b-naphthylamide	30	37
β-Glucuronidase	Naphthol AS-BI	20	37
ASD-esterase	Naphthol ASD	120	r.t.
Cytochrome oxidase	Naphthol ASLC	60	r.t.
Monoaminoxidase	Tryptamine HCl	30	37

Table 2. Clinicopathological parameters of the examined cases

Histology		Grade of malignancy	No. of cases
Lobular			1
Ductal	Non-invasive		2
Ductal	Invasive	I	9
Ductal	Invasive	II	25
Ductal	Invasive	III	9
Medullary			4
Hormonal status			
Pre-menopausal			11
Post-menopausal			39
Presence of secondaries			
Axillary lymph nodes positive			28
Axillary lymph nodes negative			22

adjacent normal epithelium. Cases with decreased activity are few (Fig. 1).

The phosphatases activity is variable. The majority of the cases (62%) show an increased activity of acid phosphatase in comparison to the normal adjacent epithelium. The activity of 5-nucleotidase is either equal (66%) or decreased (Fig. 1).

The observed activity of β-glucuronidase and MAO is also variable. Activity of NSE appears increased in a relatively larger number of cases (44%) and activity of LAP decreased in 42% of cases. Cytochrome oxidase shows either an increased or an equal activity to the adjacent epithelium in the majority of cases (Fig 1).

The activity of three enzymes, SDH, G6PDH and MAO was found significantly increased in postmenopausal women ($P<0.05$, $P<0.001$, and $P<0.05$, respectively) (Fig. 2). The pattern of a number of enzymes appears to be related to the differentiation of the tumour as this is indicated by the grade of malignancy, Activities of SDH, glutamate DH, G6PDH, alcohol DH and NADH appear decreased in grade I carcinomas and increased in more anaplastic (grade II and grade III) tumours ($P<0.05$ for each enzyme). A similar pattern shows MAO and NSE ($P<0.05$ and $P<0.005$, respectively) (Fig. 3).

Finally, cytochrome oxidase activity in-

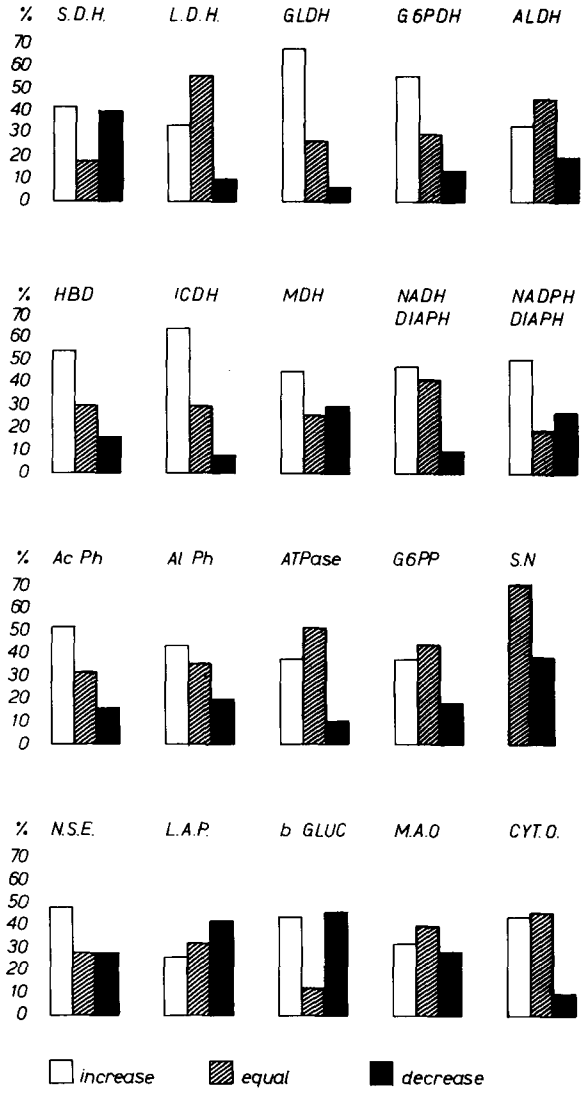

Fig. 1. Activity of the examined enzymes. Columns indicate the percentage of the tumours in which enzyme activity was lower, equal or higher than in adjacent epithelium.

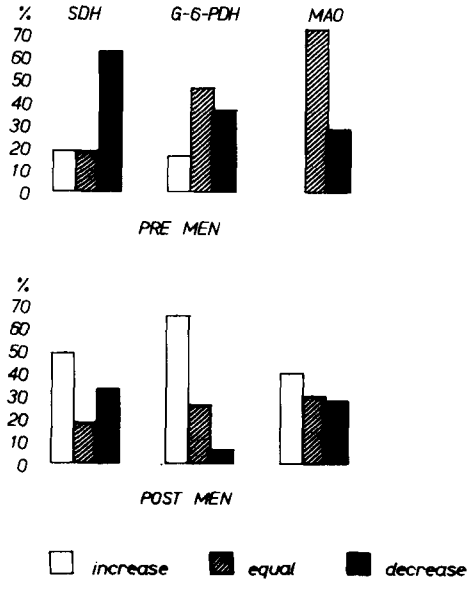

Fig. 2. Activity of enzymes related to the pre- or postmenopausal period. (See legend to Fig. 1.)

creases in cases with secondaries in the axillary lymph nodes and this observation was found statistically significant ($P<0.05$) (Fig. 4). There was no evidence that there is a relation of any of the observed enzymatic patterns to the age of the patients and the gross diameter of the tumour.

DISCUSSION

From the previous results it appears that enzymes of intermediate metabolism are increased in breast carcinomas. This is in accord with the hypothesis that the tumour cell has streamlined its metabolism in an attempt to simplify its growth and proliferation [5]. It appears also that activity of metabolic enzymes is decreased in well differentiated carcinomas (grade I).

Increased activity of LDH indicates increased activity of the glycolytic pathway and therefore of anaerobic energy production via the diaphorases. Increased activity of NADPH diaphorase is also an indication of nucleic acid biosynthesis [1]. Koudstaal *et al.* [6] found no significant differences between the enzymatic activity of two groups of breast carcinomas. Among the examined enzymes was SDH but the examined groups were grade II and grade III tumours.

Two of the examined enzymes (SDH and G6PDH) showed an increased activity in postmenopausal women. This is in accord with the observations of previous investigators [7–9], but we didn't find significant hormone dependent differences in NADPH diaphorase as was reported by Hilf *et al.* [9].

Monoaminoxidase appears also to be a hormone dependent enzyme. We didn't find any report of its activity in human breast tissue.

Increased phosphatases activity in breast and prostatic carcinoma is an exception to most human tumours. This has been related to the hormonal dependence of both these tumours [10] but there was no evidence from our results that phosphatases activity is different in women in the pre- or post-menopausal period. Acid phosphatase is not essential for the provision of energy or the production of cofactors necessary for nucleic and protein production [1] and has been reported to show a decreased activity in a number of tumours [11, 12]. Increased acid phosphatase activity has been observed in the majority of our cases and this is in accord with previous observations [10–14]. Wachstein [15] found no 5-nucleotidase activity in 13 breast carcinomas. We observed an equal activity to the adjacent

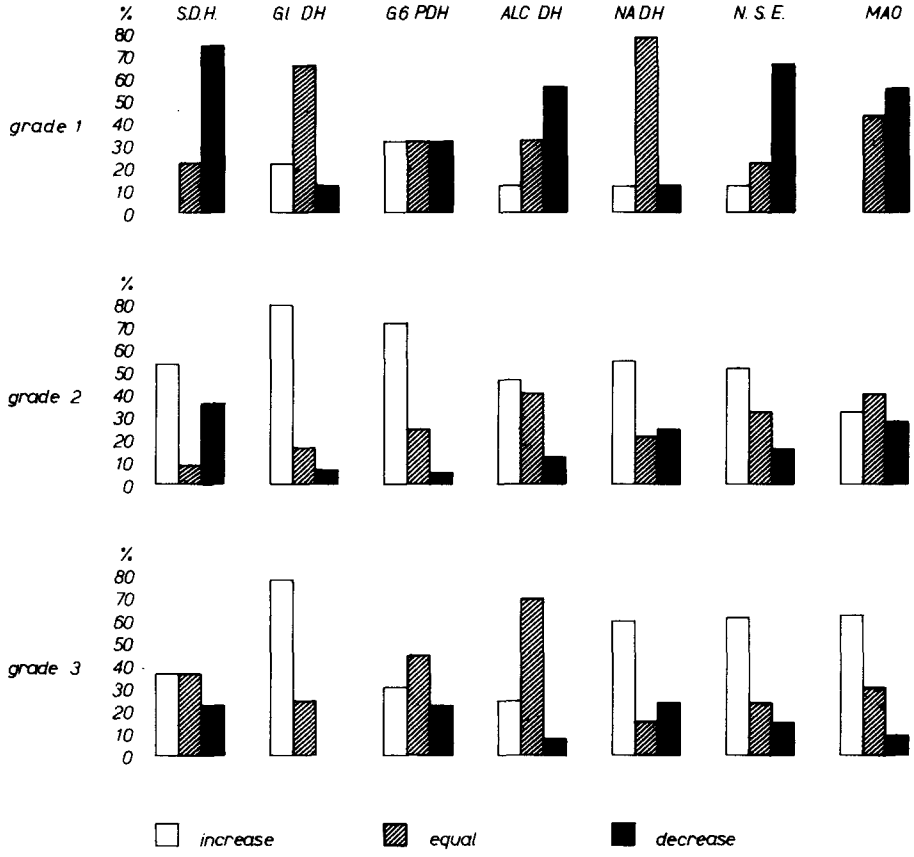

Fig. 3. Activity of enzymes related to the grade of malignancy. (*See legend to Fig. 1.*)

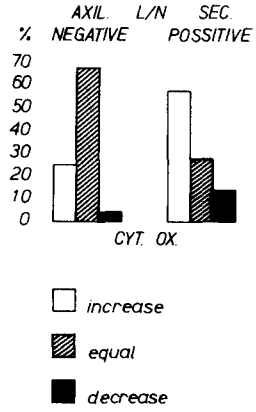

Fig. 4. Activity of cytochrome oxidase in relation to the presence of axillary secondaries. (*See legend to Fig. 1.*)

epithelium in the majority of the examined tumours (66%). The observed patterns of ATPase and alkaline phosphatase are in accord with previous reports [10, 14, 16]. From the previous results it appears as well that MAO and NSE decrease in well differentiated (grade I) tumours and increase in less differentiated. The number of cases classified as grade I was small (nine cases) but among them there were none found with increased MAO and only one with increased esterase activity, while five and six cases respectively showed a decreased activity. Whether this observation and especially whether decreased activity in grade II and III tumours can be important for the prognosis, must be a matter of further investigation and follow-up of a larger number of cases.

Reduced activity of enzymes not essential for the process of rapid growth, among them MAO, NSE and LAP, was related to the lack of differentiation of other human tumours, because these enzymes are not concerned with the provision of energy under conditions of relative oxygen lack or the production of cofactors necessary for nucleic and protein production [1]. This theory does not appear to apply in human breast carcinoma where we observed the opposite.

Esterase is reported to have decreased activity in medullary carcinomas [17]. Our material included four of these tumours, one of which showed an increased activity, two a decreased one and one an equal activity. Our results for this enzyme in all of our cases are in accord with the observations of Koudstaal *et al.* [10]. El Fiky [18] found experimentally that LAP activity is related to the diameter of

the tumour, its infiltration and the presence of lymph node secondaries. We didn't find in our material any relation of the enzyme activity to any of these parameters. The enzyme shows a decreased (43%) or equal (32%) activity in the majority of our cases and this is not in accord with the observations of Koudstaal *et al.* [10] who found the enzyme mostly increased.

We didn't find any reference of the activity of β-glucuronidase in human breast. Experimentally it was found that the enzyme increases in the rat mammary gland after oestrogen stimulation [19] but there was no evidence of relation to the presence of menstrual cycle in our material. The role of hormones in breast carcinoma is a matter of constant investigation and the presence of oestrogen receptors in tumour tissue appears to be important for therapy and prognosis. Whether the pattern of hormone dependent enzymes is of equal importance can be a matter of further investigation, especially if it is related to the presence of the above receptors. The presence of an increased cytochrome oxidase activity in tumours with axillary secondaries is not related to the grade of malignancy. It is difficult to explain and although it was found statistically significant it seems to us that it must be a matter of further investigation in order to estimate the possibility of prognostic value of this observation. From the previous results it appears to us that enzyme histochemical pattern of human breast carcinoma in relation to the follow up of the cases now in progress, may provide us with data important for the prognosis and the biological behaviour of this tumour.

REFERENCES

1. F. McGinty, G. S. Delides and D. Harrison, The significance of enzyme histochemical patterns in carcinomas of the large intestine in man. *Gut* **14,** 502 (1973).
2. R. A. B. Drury and E. A. Wallington, *Carlton's Histological Technique*, p. 239. Oxford University Press, London (1967).
3. J. Chayen, L. Bitensky, R. G. Butcher and L. W. Poulter, *A Guide to Practical Histochemistry*, p. 12. Oliver and Boyd, Edinburgh (1969).
4. H. J. G. Bloom and W. W. Richardson, Histological grading and prognosis in breast cancer. *Brit. J. Cancer* **11,** 359 (1957).
5. W. E. A. Criss, A review of isoenzymes in cancer. *Cancer Res.* **31,** 1523 (1971).
6. J. Koudstall, B. Makkink and S. H. Overdiep, Enzyme histochemical pattern in human tumours. II. Oxidoreductases in carcinoma of the colon and the breast. *Europ. J. Cancer* **11,** 111 (1975).
7. H. Jensen, Fibro-adenomatosis and breast carcinoma. Enzyme histochemical study. *Acta path. microbiol. scand.* (*sect. A*) **78,** 421 (1970).
8. J. L. Daechnfeldt and M. Schulein, High affinity oeastradiol receptors and the activity of a glucose-6-phosphate dehydrogenase and lactose synthetase in mammary carcinoma of postmenopausal women. *Brit. J. Cancer* **31,** 424 (1975).
9. R. Hilf, R. Ickowicz, J. C. Bartley and S. Abrahams, Multiple molecular forms of glucose-6-phosphate dehydrogenase in normal, preneoplastic and neoplastic mammary tissues in mice. *Cancer Res.* **35,** 2109 (1975).
10. J. Koudstaal, B. Makkink and S. H. Overdiep, Enzyme histochemical patterns in human tumours. I. Hydrolytic enzymes in carcinoma of the colon and the breast. *Europ. J. Cancer.* **II,** 105 (1975).
11. M. M. Nachlas and M. J. Hannibal, Histochemical observations of the polyp-carcinoma sequence. *Surg. Gynec. Obstet.* **112,** 534 (1961).
12. B. Czernobilsky and K. C. Tsou, Adenocarcinomas, adenomas and polyps of the colon. *Cancer* (*Philad.*) **21,** 165 (1968).
13. H. Fanker and B. Barker, Histochemistry of breast desease. I. Phosphatases. *Arch. Path.* **67,** 293 (1959).
14. R. G. J. Willighagen, Histochemisch onderzoek van de aktiviteit van alkalische en zure fosfatase in normaal en in pathologisch verandered menselijk weefsel. Dessertatie Rejksuniversiteit Leiden. (1960).
15. M. Wachstein, Histochemistry of enzymes in tumours. *Handbuch der Histochemie*, Vol. VII, 2nd part. Gustav Fisher, Stuttgart (1962).
16. J. M. Drennan, Alkaline phosphate in breast carcinoma. *Brit. J. Surg.* **39,** 458 (1952).

17. P. J. Melnick and K. Bullock, Histochemical studies of breast neoplasms. *Amer. J. Path.* **35,** 706 (1959).
18. S. M. El Fiky, Ultrastructural cytochemistry of the enzymes associated with mouse mammary tumour virus (MTV). *Acta histochem. (Jena)* **42,** 126 (1972).
19. J. H. Cutts, Enzyme activities in the regressing oestrone induced mammary tumours of the rat. *Cancer Res.* **33,** 1235 (1973).