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Assessment of Hormone Sensitivity of Human Mammary Tumours by Diffusion Chamber Culture

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Controversy remains regarding the role of estrogens and prolactin in the induction and maintenance of mammary tumours (2, 6, 11, 15, 19). It is not clear whether estrogens act synergistically with prolactin to promote tumour growth at the tumour tissue level, or simply stimulate prolactin which in turn enhances tumour growth (7, 13). However, most of these reports are from dimethyl benzanthracene induced mammary tumours of rat, which serve as a model for human tumours.

Differences exist in the hormone responsiveness of human mammary tumours, hence determination of hormonal sensitivity is an extremely essential prerequisite for hormonal intervention in therapy. The organ culture studies and receptor analysis provide information but the *in vitro* environment can never simulate the highly controlled and integrated endocrine system of the intact organism.

On the other hand, receptor analysis is an expensive and elaborate procedure unsuitable for clinical laboratories. The diffusion chamber culture technique originally described by Algiers (1) is currently used in blood and bone marrow proliferation (8-10, 18), immunological studies (4) and assessment of drug sensitivity of tumours (3), is a model system of *in vivo* culture of human tumours in animal host. This technique permits hormones and other humoral molecules into the culture but prevents the passage of cellular structures, hence protects heterografts from being rejected. In the present study this technique has been introduced to assess hormone responsiveness of human tumours in altered endocrine conditions of the host which include 1) induction of hyperprolactinemia by pituitary stalk section, 2) removal of primary source of estrogen by bilateral oophorectomy 3) or both.

MATERIALS & METHODS

Diffusion chambers were constructed from non-toxic Plexiglass rings to

which millipore membrane filters 0.45μ were fixed with non-toxic adhesive cement with the help of assembly tool. Chambers were washed and sterilised with solutions of streptomycin 100μg/ml, kanamycin 100μg/ml and amphotericin 25μg/ml. After aseptic collection of tumour, the tissues are minced and suspended in sterile balanced salt solution and cells were dissociated with collagenase solution (0.3mg/ml in balanced salt solution) and centrifuged at a moderate speed. The pellets were resuspended in 199 media with antibiotics and 5% foetal calf serum. Viable cell counts were done by dye exclusion techniques, and required amount of cells were loaded in each chamber. The chambers were implanted intraperitoneally 4/rat in 50 female Wistar rats which were operated 2 weeks earlier and grouped into 4 groups, 12-14 rats/group. 1) Intact controls; 2) Pituitary stalk sectioned; 3) Pituitary stalk sectioned and oophorectomised; 4) Oophorectomised. Pituitary stalk section was done by the subtemporal route and a minute steel plate was inserted between the cut ends to form a permanent barrier. A total number of 14 human adenocarcinoma of breast have been tested. 12 Tumours have been grown in intact rat, all 14 in pituitary stalk section group, 12 in stalk section plus oophorectomised group and all 14 in the oophorectomised group. Of these the number of diffusion chamber with active growth has been mentioned. The rest was discarded due to infection in the culture. A separate rat was used for testing each tumour. After 7 days the chambers were recovered and following treatment with 0.5% Pronase the cells were aspirated and viable cell counts were done and smears were made for cytochemical and cytological studies. Cellular succinic dehydrogenase activity was determined by the technique of Nachlas *et al* (17)., using nitro blue tetrazolium as the electron acceptor.

RESULTS

The adenocarcinoma cells grew in the diffusion chamber culture for varying lengths of time upto a period of 21 days. Considering the cytological and morphological characteristics of the cells the experiments following endocrine ablations were restricted to 7 days where cells were more or less uniform in size and shape with prominent nucleoli. Mitotic figures were occasionally present.

Elevation of prolactin by pituitary stalk section induced growth stimulation in 83.3% tumours in the diffusion chamber culture compared to 11.1% in the intact controls. Prolactin elevation together with the withdrawal of estrogen produced a mixed response, 36.3% tumours showed growth stimulation and

45.4% tumours displayed normal growth pattern. Oophorectomy alone caused inhibition of 45.4% tumours and activation in only 9% tumours. The mean cell count of human adenocarcinoma, grown in diffusion chamber culture increased from 7.4×10^7 in the intact to 18.3×10^7 in the stalk sectioned group. It considerably dropped to 3.1×10^7 in the diffusion chamber culture in oophorectomised condition. Similarly the mitotic index also showed a parallelism, increasing in the stalk sectioned group and decreasing in the oophorectomised group (Table 1 and 2).

Table 1. *Growth Response of Human Adenocarcinoma in Diffusion Chambers in Rats with Endocrine Ablation.*

Group	No. of tumors evaluated	No. of rats implanted	No. of active culture	Growth Response %		
				Inhibition	Normal	Activation
Intact	12	12	9/12	—	88.8	11.1
Pit. stalk section	14	14	12/14	—	16.6	83.3
Pit. stalk section + oophorectomy	12	12	11/12	18.1	45.4	36.3
Oophorectomy	14	14	11/14	45.4	45.4	9

Table 2. *Mean Viable Cell Counts and Mitotic Indices of Human Adenocarcinoma Cells Grown in Diffusion Chamber Culture in Rats Following Endocrine Ablation.*

Group	Mean Viable Cell Count $\times10^7$/ml	% of Change of Cell Count Over Control	Mitotic Index
Intact	7.4±0.742 *	—	2.2 ±0.08 *
Pit. stalk section	18.3±1.387	148	4.8 ±0.174
Pit. stalk section+ oophorectomy	8.5±1.2	14.86	2.6 ±0.276
Oophorectomy	3.1±0.126	−58	0.58±0.02

* Standard Error

Succinic dehydrogenase activity was present in moderate amounts in the human adenocarcinoma cells. Following diffusion chamber culture the enzyme

activity was slightly inhibited. In the pituitary stalk sectioned group the mean percentage of cells showing high reaction for this enzyme increased from 13.2 to 35.8, indicating high growth rate of the tumours. In the stalk sectioned and oophorectomized groups the mean percentage of cells showing high succinic dehydrogenase activity was also increased over the control and intact values. Following oophorectomy the mean percentage of cells showing high and moderate activity also fell considerably and in this group 55.8% cells displayed low activity of the enzyme (Table 3).

Table 3. *Mean Percentage of Cells Showing Different Grades of Succinate Dehydrogenase Activity of Human Adenocarcinoma Cells Grown in Diffusion Chamber Culture*

Group	% of Cells Showing Different Grades of Sdh Activity		
	High 3+4+	Moderate 2++	Low+−±
Control	16.8	65.3	17.9
D. C. culture Intact	13.2	52.6	34.2
Pit. stalk section	35.8	49.7	14.5
Pit. stalk section+ oophorectomy	20.2	51.6	28.2
Oophorectomy	5.9	38.3	55.8

DISCUSSION

It has been reported earlier that transection of pituitary stalk or placement of electrolytic lesions in the median eminence blocks of the prolactin inhibiting factor PIF from the hypothalamus inducing a sharp rise of serum prolactin within 30 min. with a simultaneous reduction of all other pituitary hormones. This elevated level is significantly retained to higher than normals for at least 5 months (16). Plasma estradiol was significantly decreased following both transection of pituitary stalk and oophorectomy. Following transection of the pituitary stalk plasma prolactin levels was elevated 4 times the control value of 68.5mg/ml. Oophorectomy also caused a significant reduction of prolactin level. Electron microscopic studies of the pituitary prolactin cells also revealed high synthetic activity following transection of pituitary stalk and reduction of synthetic activity following oophorectomy (unpublished).

Our results following induction of such hyperprolactinemia by pituitary stalk section and withdrawal of estrogen by bilateral oophorectomy indicate

that elevation of prolactin stimulates mammary tumour growth in majority of the cases. It is evident from our studies that there is considerable overlap in the hormone sensitivity of human tumours, and the same tumour may be dependent on estrogen or prolactin or both. Our results also simulate the findings on the DMBA induced rat mammary tumours in similar situations, where transection of the pituitary stalk (5) or transplantation of multiple pituitaries (20) significantly stimulated tumour growth. The regression of the mammary tumours in the oophorectomised and stalk sectioned and oophorectomised rats indicate that elevated prolactin is not by itself sufficient to maintain mammary tumour growth, apparently estrogens are also essential. The estrogen and progesterone receptor analysis which is the most recommended technique for prediction of hormone sensitivity in therapy reveals that about 80% cases are estrogen and progesterone receptor positive, of which about 55% cases respond to oophorectomy (12, 14). We have observed regression of 45.4% tumours in the diffusion chamber culture in oophorectomised rats. The figure although slightly higher shows a good conformity with the results obtained from different hospitals with or without receptor assay.

The viable cell count, the mitotic index and cellular succinate dehydrogenase activity have been analysed for assessment of growth. All these three indices show a good parallelism, with enhancement or regression of tumours.

The results so far obtained promise immense possibility for the diffusion chamber culture technique to stand as a simple and economical tool for assessment of hormonal sensitivity of human tumours which is essential for monitoring endocrine therapy, especially in laboratories where facilities of receptor assay are not available.

SUMMARY

Human adenocarcinoma cells have been grown by the diffusion chamber culture technique in Wistar rats in intact and experimentally altered hormonal conditions. These include induction of hyperprolactinemia by pituitary stalk section, removal of primary source of estrogen by oophorectomy or both. Prolactin elevation by pituitary stalk section caused growth enhancement in the diffusion chamber culture in most of the tumours, while oophorectomy caused regression in about 45.4% tumours. Pituitary stalk section and oophorectomy showed a mixed response. Viable cell count, mitotic index and cellular succinic dehydrogenase activity showed a parallelism with growth enhancement. The application of diffusion chamber culture may be suggested as a simple and economical tool for the assessment of hormonal sensitivity of human tumours.

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