A multidisciplinary Symposium took place in Portofino (Italy), organized by the ScientIfic Institute for Study and Treatment of Cancer (Genoa) with the sponsorship of the Italian League for Struggle against the Cancer. Presidents of the Congress were Prof L. SANTI *and* G.E. SERRA *(Genoa). It was devoted to Contact Thermography in the study of breast cancer relatively to its biology, diagnosis andfollow-up. The contribution of Contact Thermography (C.T.) has been discussed along with epidemiologic, immunopathologic and histologic considerations. The role of C.7: versus the other diagnostic techniques in the various fields of interest has been debated, namely: the screening of asymptomatic women, the characterization of a lesion in symptomatic women, the follow-up.*

The Symposium wasfollowed with great attention by the over250 delegates, being they Radiologists, Oncologists, Gynaecologists, Surgeons, Biologists and Immunopathologists.

The multidisciplinary approach of CT. in the study of breastpathology, as presented at the Congress, achieved great interest. «Acta Thermographica» has therefore considered it useful to devote the 1981 issue to the publication of the most important papers presented there.

1. GENERAL REMARKS

The clinical significance of estrogen receptors assay in primary breast cancer

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Summary. The Authors refer their personal experience in the estrogen receptors (ER) assay. The clinical value of the positivity and negativity of the ER assay is discussed, emphasizing the interesting relationship between the progressive ER concentration and the remission probability. The value of the ER assay is emphasized in order to establish the appropriate hormonal treatment and to choose the most reliable therapy; nevertheless it is not useful as prognostic factor.

Key words: breast cancer; estrogen receptors assay.

A) INTRODUCTION

Hormone dependence designates a property of certain breast cancers which regress after various endocrine manipulations. It is impressively illustrated by the following **case report.**

Supported by a grant from the <<Fonds CancCrologique de la Caisse Generale d'Epargne et de Retraite de Belgique>> and by grant n° 5RlOCA 11488-10, awarded by the National Cancer Institute, DHEW.

A 43 yrs old female was admitted to the Department of Surgery of the Jules Bordet Institute in January 1958. A small tumour had been present for yrs in her right breast but had started developing rapidly since one yr. On admission, it formed a mass, 10 cm in diameter, which extensively ulcerated the skin (Fig. 1 A). Skeletal survey disclosed a large metastatic os-

teolytic lesion of the 12th thoracic vertebra. Biopsy of the breast mass showed a poorly differentiated ductal carcinoma. Bilateral oophorectomy and adrenalectomy were performed as a two-stage procedure. Rapid improvement ensued with progressive regression of the mass and healing of the ulceration that was complete in 1960 (Fig. 1 B). A deep biopsy at the site

B) HORMONE DEPENDENCE

The property of hormone dependence was largely unpredictable until the discovery of the Estrogen Receptors (ER) and the demonstration of their presence in tumour biopsies from patients responding to endocrine therapy. Aim of this report is to define what ER are and how they can be used to predict the success of endo-

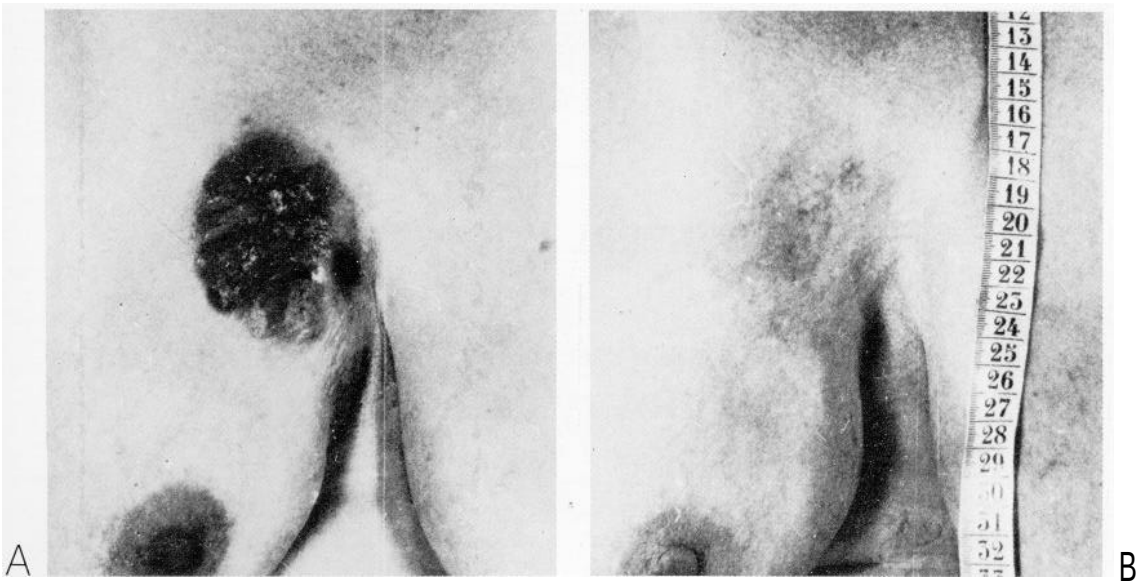


Fig. 1 A-5. Fourty-three-year old woman exibiting a large ulcerated tumour of the right breast(poorly-differentiated ductal carcinoma). A) Before treatment (1958). B) Two yrs after bilateral oophorectomy-adrenalectomy (1960). <<Healing)> of the tumour (negative deep biopsy at the site of the former mass). *(Courtesy of Dr.* P. DOR *who performed the endocrine surgical ablative procedure).*

of the former mass proved negative. The osteolytic lesion of the spine totally recalcified and never recurred. The complete remission lasted 19 yrs until January 1977 when a small superficial tumour reappeared on the pigmented scar of the right breast. After resection, this tumour exhibited the same histologic feature as the initial lesion. Of note, the estrogen receptors assay was found negative. This suggests that the recurrent tumour developed, possibly by cell selection, from a hormone-independent cell clone present in the initial lesion. Such a spectacular therapeutic success is only rarely achieved, but it serves to illustrate that similar results may occur, although often to a lesser extent, in about one third of all breast cancers.

crine treatments in women with advanced breast cancer. The potential usefulness of performing an ER assay on the primary breast cancer at the time of mastectomy will be focused.

1. Criteria of response

In the present report, the term <<remission>>, as applied to the advanced stage of the disease, means an objective regression of all visible tumour masses by at least one half of their <<surface>> (product of 2 perpendicular diameters) and no new lesion appearing. For deep visceral lesions, the criterion is a 50% decrease in the largest measurement.[3] A clear definition of the

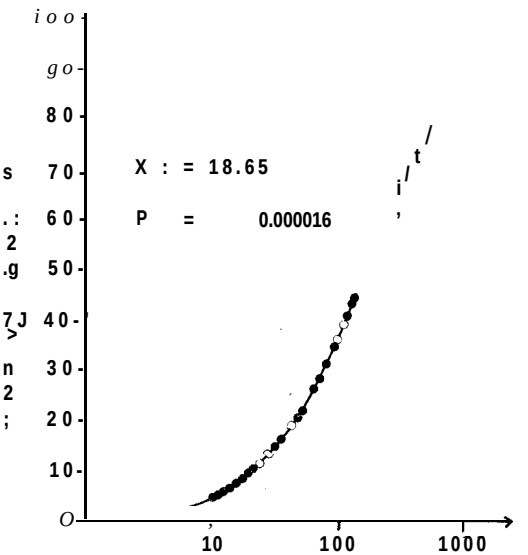
criteria of <<tremission>> is mandatory and the one summarized above, based on objective regression of tumour masses, is now the one adopted almost worldwide.

2. Estrogen receptors (ER) assay

The method used is a classical one based on the measurement of bound ^{3}H-estradiol after incubation of the tumour cell soluble fraction (cytosol) with increasing amounts of the labelled steroid, and removal of the unbound steroid with dextran-coated charcoal (DDC).[6]

CI RESULTS

1. There is now general agreement on the usefulness of the ER assay in predicting, in advanced breast cancer patients, the likelyhood of achieving an **objective response** to any of the various modalities of endocrine treatments presently available. Thus, ER-positive cases will respond in a proportion of 50-60% while the rate of response will be only 5-10% in ER-negative cases. These results are however dependent on the sensitivity of the ER- assay and on how ER positivity is defined. When sensitive methods are used,.like in the personal facilities, some 80% of the tumours are ER positive. It ensues that the rate of reponse to endocrine treatments in these cases drops to a level which is close to that obtained in the general population of patients, namely 30%. In view of the very large variation in ER concentration among ER-positive tumours, ranging from 3 to more than 1000 femtomoles/mg tissue protein, it is possible that the ER concentration might be positively associated with the rate of response to endocrine treatments. If this were so, this parameter would be more useful as'a predictor of response than the mere qualitative distinction between ER-positive and ER-negative cases. In order to test this hypothesis, a study was carried out in a series of patients with inoperable, recurrent, or metastatic breast cancers, whose responses to an endocrine treatment could be assessed and who had a biopsy done for histology and ER determination before the treatment. Fourty-nine treatment cycles were available in 48 patients. Statistical analysis, using Cox's linear logistic regression model, showed that the ER concentration was indeed closely related to the therapeutic response ($P < 0.0001$) and proved superior in this regard to the qualitative distinction (ER-positive vs. negative). The curve represented in Graph. 1, illustrates this relationship and shows that the response probability is a continuous function of the receptor concentration; [7] it can be used by the biochemist who performs the ER assay in order to provide the clinician with an estimate in percent of the probability of his patient to respond to an endocrine treatment. Furthermore, this relationship reveals more general biological and therapeutic implications insofar, as it suggests that breast cancers



Graph. 1. Relationship between ER concentration and probability of response to endocrine therapy. The experimental data were analyzed using the linear logistic regression model. The model that best fits the data is $\ln P/(1-P) = 5.504 + 2.488\ X$, where P is the probability of response and X the logarithm of receptor concentration. The figure is a graphic representation of this formula. Using the linear logistic regression model, the symbols on the curve give the expected probabilities of response calculated for each of the 49 patients from their receptor concentration. (0) cases of remission; (●) non responders.

are distributed along a continuous gradient of hormone dependency that can be assessed by ER content. All patients might in fact benefit from hormonal treatments, although in vary-

ing degree, and endocrine therapy always has a role to play in breast cancer treatment. For the restricted group of highly hormone-dependent cases, endocrine therapy might be sufficient, leading sometimes to dramatic and prolonged responses, as in the case reported above. For the other, less hormone-dependent cases, an associated chemotherapy and endocrine therapy is the approach most likely to provide a high rate of rewarding responses.

2. An important question, is to know whether one should **routinely perform** an **E W assay** on the primary lesion at the time of mastectomy. This attitude has potentially useful applications for selecting an appropriate adjuv*ant treatment,* for providing an assessment of the *hormone dependence* of the recurring tumour when the disease has reached the advanced stage and for supplying a *prognostic evaluation* of the subsequent course of the disease. These *potential applications* will be discussed in succession.

a) There are several ongoing trials of endocrine *adjuvant treatment* involving the ER assay as a basis for the selection and classification of the patients. One such trial has now been reported and illustrates the usefulness of this procedure.' In this study, CMF chemotherapy (cytoxan, methotrexate and fluororacil), given as an adjuvant treatment after mastectomy for breast cancer, was compared to CMF plus the antiestrogen tamoxifen (CMFT). It was found that in ER-negative patients there was no difference between the treatment groups whereas in ER-positive patients CMFT was much more effective in delaying recurrence than CMF alone ($P = 0.0176$). These results indicate that endocrine treatments may prove useful as part of an adjuvant therapy after mastectomy and that the ER assay may serve to define the subset of patients who are likely to benefit from these treatments.

b) The second potential usefulness of performing an ER assay on primary breast cancer is to provide information that will serve later on for the *selection of an appropriate therapy* when the disease has progressed to the advanced stage. In order to study this possibility, ER values in the primary tumour were compared with those found in biopsies of recurrent disease in a series of patients in whom the information were available. In post-menopausal patients who received no intercurrent treatment between the 2 consecutive assays, there was a highly significant correlation between them ($P < 0.0001$). As far as the pre-menopausal patients are concerned, their number was too small to allow firm conclusions to be drawn. There was however a suggestion that the results might be less concordant.

The influence of intercurrent treatments was also studied. For that purpose, either primary cancer and recurrent disease or metastatic lesions taken sequentially were compared. When radiotherapy was given post-operatively or for recurrent disease, biopsies of metastatic lesions arising later on in the irradiated field gave ER assays that were less consistent with the initial values. The same was true but to an even greater extent, when cytotoxic chemotherapy was given between the 2 assays. It is striking, however, that when a 3rd biopsy was assaied long after the end of the treatment, ER were again closer to the initial value. The greatest discrepancies occurred when the antiestrogen tamoxifen was given alone or as part of a combined treatment between the 2 assays. The values in the second assay were uniformely much lower than in the first and were often zero or close to zero. This was always so when the time elapsed between discontinuation of tamoxifen and the biopsy was less than 24 months. Most remarkably, however, when the time lapse exceeded 24 months, the second value was again close to the initial one. In fact, it was lower in only 2 determinations but higher in 6.

In conclusion, in post-menopausal patients, ER determination in primary breast cancer provides an estimate of the hormone dependency of the tumour that probably remains valid during the whole course of the disease. The administration of intercurrent treatments, especially those comprising tamoxifen, may change the ER content of a biopsy taken afterwards although the change seems most often transient. The nature of this change has not been investigated.

c) The third potential application of an ER assay in primary tumour is related to its alleged *prognostic value.* There have been several reports claiming that ER-negative cases had a

worse prognosis than ER-positive ones both in terms of relapse-free survival and total survival. These reports have been criticized for several reasons, and challenged in a study characterized by a relatively long follow-up period, i.e. 90 months for the earliest patient.[5] In this study, there was no prognostic influence of the ER status of the primary tumour regardless of post-operative treatment. Since personal experience in measuring ER in all primary breast cancers started very early, namely in 1972, it was of interest to analyse the potential prognostic value of the ER status in a series of 364 patients uniformely treated by the MADDEN modified radical mastectomy. Adjuvant chemotherapy was given to a majority of the N+ cases only since 1975 and the study did not include the patients treated after 1978. It was found that the ER status, whether negative (< 10 femtomoles/mg tissue protein) or positive, bore no relationship to the axillary nodal status, whether involved or not. Various parameters of known prognostic value as well as the ER status were studied in relation to the disease-free survival and total survival. The survival curves drawn according to the method of KAPLAN-MEIER, were statistically analyzed by the methods of Peto and PIKE [8] and of GEHAN[2] The size of the tumour, the axillary nodal status (O-3 vs. > 4 positive nodes) and the histological grading of malignancy' where found to exhibit a very significant prognostic influence on survival, which is consistent with the data in medical literature. In contrast, the ER status was not associated with a significant difference in survival by whatever test statistic used. The ER-negative curves are somewhat steeper than the ER-positive ones early after mastectomy but they cross each other after about 4-5 yrs. The ER status had also no prognostic influence when considered in the subsets of N- and N+patients. In conclusion, in the large series of patients followed up for a long period after mastectomy, the ER status was of no prognostic value as to the post-operative course of the disease.

D) DISCUSSION

According to the presented data, it is most useful to perform an ER assay in all primary breast cancers subjected to surgical ablation. The ER assay provides, in post-menopausal patients, a reliable estimate of the hormone dependence of the tumour that remains valid during the whole course of the disease. Intervening treatments, especially those involving tamoxifen, tend to change the ER values but only in a transient manner. The situation is less clear in pre-menopausal patients since more. variability was observed, possibily as a result of the cyclic hormonal variations occurring in these patients. It is likely that the ER assay should then be performed at a fixed time with respect to the menstrual cycle. Further investigation is needed to settle this question. In contrast to the valuable information provided by ER assay in terms of hormone dependence, it was disappointing to observe that, in the personal large series of patients observed for prolonged periods of time, ER were devoid of prognostic significance with regard to the length of disease-free survival or total survival.

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Ionizing radiations: DNA damage, mutagenicity and morphological transformation in mammalian cells. Carcinogenicity in rodents and in human

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Summary. X-rays induce single and double strand breaks and cross links in DNA. They are mutagenic in procariotes and mammalian cells and induce transformation in cells grown in vitro. For the above activities, at equitoxic doses they are about 10 times less potent than the most active chemical agent. A similar relationship seems also to hold for their carcinogenic potency in inducing tumours in small rodents. For the same dosage, a much longer latency in tumour induction exists in human beings in respect to rodents. This suggests that more steps are involved in the multistep process of carcinogenesis in human beings in respect to small rodents.

Key words: X-rays, DNA damage, mutagenesis, small rodents carcinogenesis, human carcinogenesis.

A) INTRODUCTION

Ionizing radiations are extremely heterogeneous in terms of energy (from 10^2-10^4 eV for diagnostic X-rays to 10^9 eV for the highest energy radiations utilized in radio-therapy).[26] Moreover, ionizing radiations include not only photons but also particles of different masses, charges and energies.

Only the soft and hard X-rays will be discussed; this especially in the prospective of tumours they may induce on mammary tissue of rats and women. Initial effect of X-rays is the stripping of electrons from all kinds of molecules. Water is by far the most abundant molecule in the biological material; in consequence, reactive radicals generated by the interaction of X-rays with H_2O are the major source of reactive materials. The different types and importance of these initial products will not be discussed here. It is also assumed as widely accepted that the interaction of the above mentioned radicals with DNA is probably the most important cause of their gene-toxic effects.

B) DNA MODIFICATION AFTER X-RAYS EXPOSURE

1. According to general opinion **2 major effects** seem to be induced on cellular DNA following X-rays (energy - 2.50 kV) exposure: a) *single stranded breaks* in DNA of short half-life (- 20 mins); *b) cross linking DNA-DNA* and DNA-chromosomal proteins. Since the first type of damage disappears very rapidly and as cross-linkings are strong inhibitors of polymerases progression, it is assumed that damages of the second type are not only more lasting but also biologically much more important. Fig. 1 shows a typical technical approach which is capable of evidentiating both alkaline DNA. fragmentation and cross-linking effects of X-rays (energy 200 kV, doses 500-3000 rads) on the same cells.[4] The asymptomatic behaviour of the late elution fractions shows the presence of cross-links.[24]

2. The **main effects** at DNA level are of 2 types: a) *toxic effects* (inhibition of polimerases progression, inhibition of DNA and RNA synthesis and consequent cell death); *b) damages* that cells can bear, but with miscoding effects that will manifest themselves under the form of mutations and/or the hypothetical first step in carcinogenesis. Fig. 2 shows the mutagenic potency of X-rays (energy 200-300 kV) at equitoxic doses,[5, 6, 7, 8, 9, 14, 16, 27] in comparison with the mutagenic effect of typical mutagenic chemicals.[22, 23] Fig. 3 shows a similar situa-

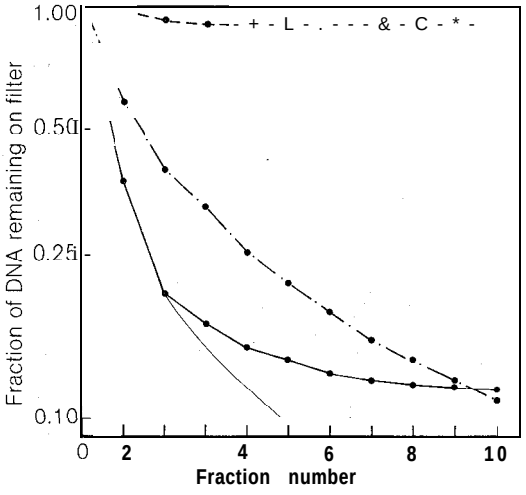

Fig. 1. Patterns of rat liver DNA after in viva treatment with 10 mg/kg of dimethylnitrosamine (−·−·−·−) and after in vitro treatment of liver nuclei with 3000 rads (-1. The slight line represents the expected slope of alkaline elution in absence of cross linkings. Control DNA (- - - -). The elution technique was performed as reported in".

Fig. 4 shows the carcinogenic potency of X-rays at equitoxic doses," in respect to the carcinogenic potency of other typical carcinogens.[25]

3. At this point it is interesting to compare type, frequency and latency period of tU-**mours** in rats and humans, for radiation dosages of the same order (200-700 rads). A 750 rads dose (energy 260 kV) induced in female Sprague Dawley rats approximately 9/21 mammary tumours with an average latency time of 11 months.[15] In female breasts, 100-300 rads dose gave the following results: [1] an approximate 2% increase of cancers in comparison with a control group, with an average latency of 24 yrs. Data were obtained from women submitted to repeated fluoroscopic examinations, because of a pneumothorax treatment due to tubercolosis.

It seemed interesting to comment on the possible significance of frequencies and very

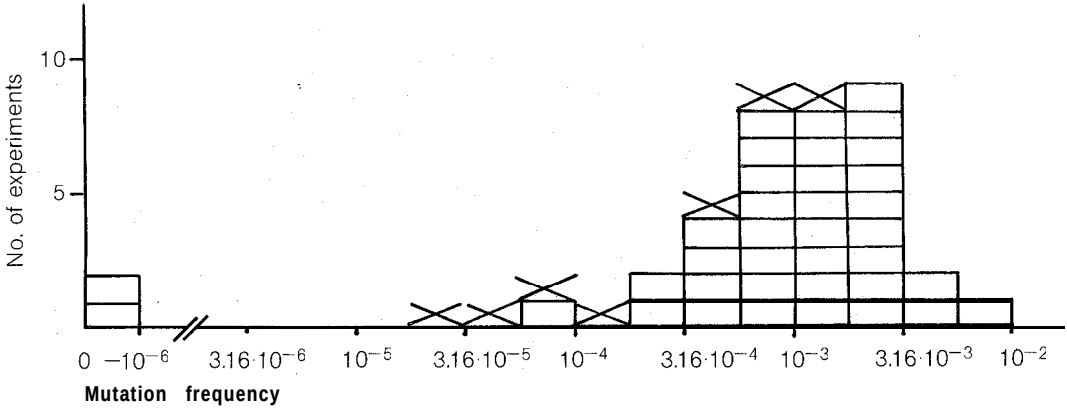

Fig. 2. Mutation frequencies in rodent cells in vitro at equitoxic doses: for a series of chemical carcinogens (0) already reported in [8,10]; the same for X-rays (X) treatment (normalization of literature data).

tion for morphological transformation in vitro.[3,10,19,22,23] Both Fig. 2 and Fig. 3 clearly suggest that X-rays, at equitoxic doses, are both amongst the most potent mutagens or transformation agents.

As expected, X-rays are not only capable of inducing mutations and morphological transformations in cells grown in-vitro but also tumours in small rodents.

different latencies of the mammary tumours induction in the 2 species.

The probability for a single cell to make a single «transformation» step (in the framework of the multi-step theories of oncogenesis) will be (as a first approximation):

$$p = (R + Bt) \qquad (1)$$

where «R» is the probability of «transformation» in-

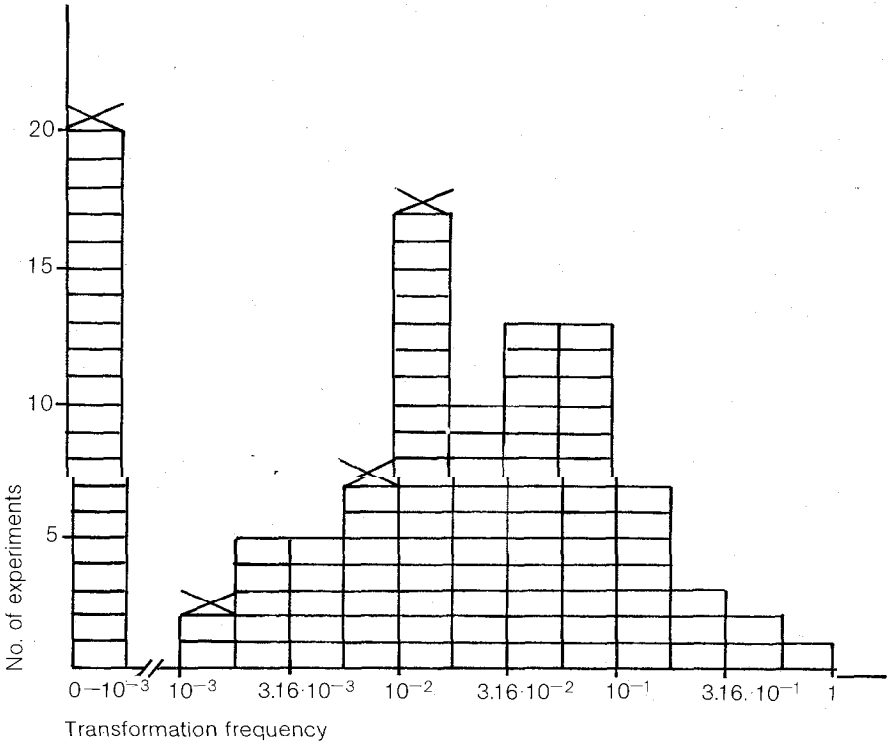

Fig. 3. Transformation frequencies in rodent cells in vitro, at equitoxic doses: for a series of chemical carcinogens (0) already reported in [8, 10]; the same for X-rays (×) treatment (normalization of literature data).

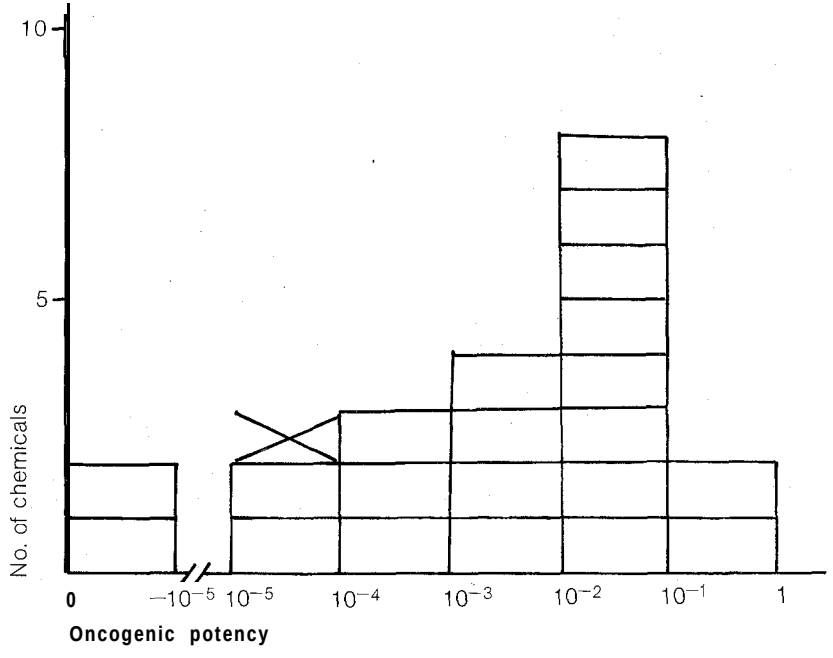

Fig. 4. Oncogenic potency in rodents: for a series of chemical carcinogens (□), from a submitted manuscript; for X-rays (×) treatment (normalization of data of [23]).

duced by the X-ray treatment, «B» the background probability and «t» the time in yrs («R» will be tentatively assumed in the order of $5 \cdot 10^{-3}$ and «B» in the order of 10^{-4}: typical transformation frequencies for treated and control cells in vitro). It follows that, for «n» steps,

$$p = (R + Bt)^n$$

In small rodents «n» is often assumed $\cong 4$.[20] If «p_o» is the probability of a single cell of remaining normal, then:

$$p_o = 1 - (R + Bt)^n \qquad (2)$$

Obviously not a single cell but many cells are being dealt with. Assuming an «m» number of 10^8 target mammary cells in a female rat and 10" target mammary cells in a woman (precise estimation is absolutely non important for these considerations), the probability «p_o» that zero cells will become malignant will be:

$$p_o = (1 - (R + Bt)^n)^m \qquad (3)$$

which, with few passages becomes:

$$p_a = e^{-(R + Bt)^n \cdot m} \qquad (4)$$

For a female rat, introducing into the place of «R», «B», «n» and «m» the previous typical values («t»,

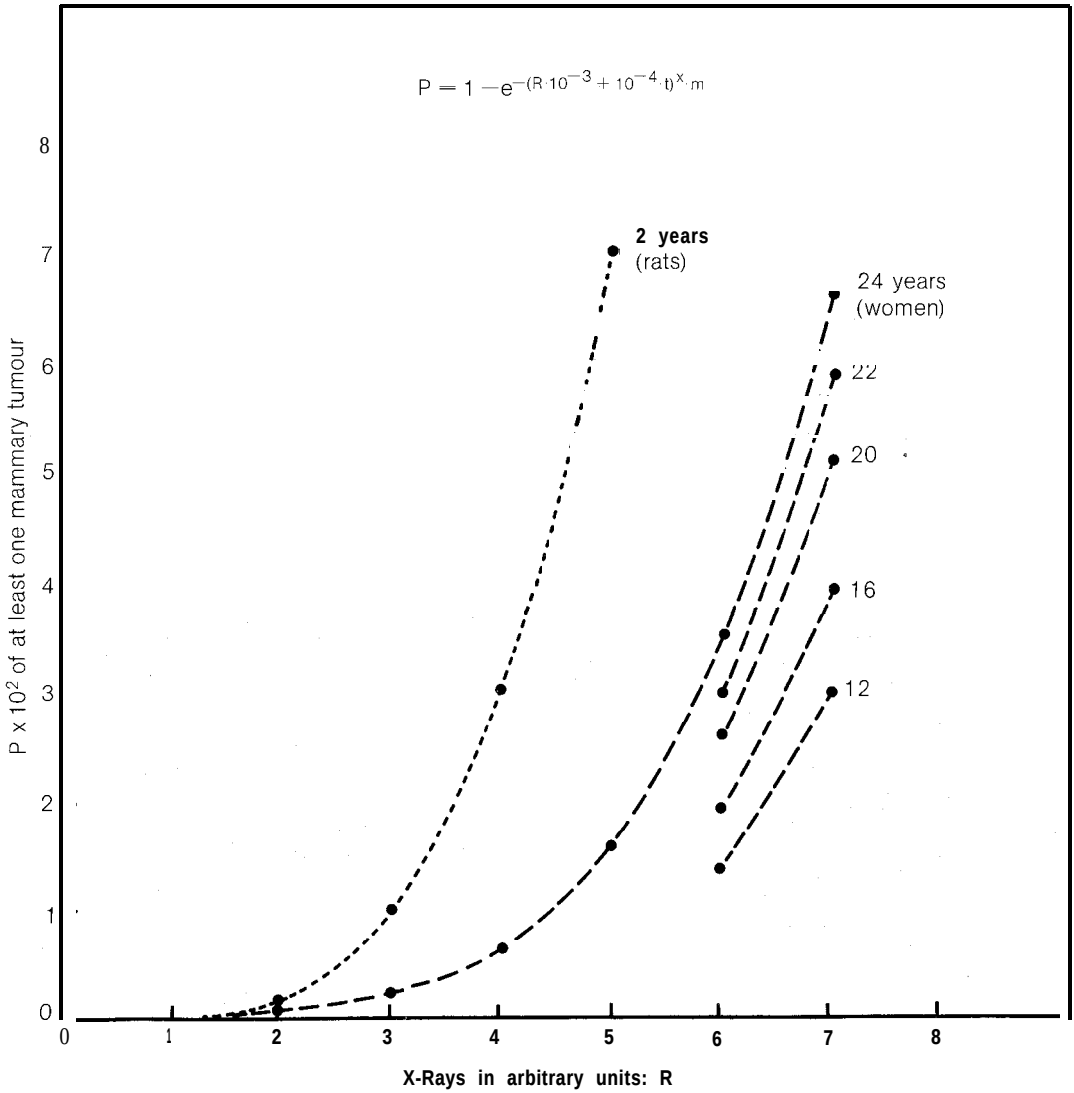

Fig. 5. Tumour frequency in women and female rats, as a function of X-ray? dosage (in arbitrary units). Graphs according to the formula on top of the figure. The formula reflects a multistep model for chemical carcinogenesis. The theoretical model is in reasonable agreement with data for women[1] and for rats[15].

referred to[15] was assumed ≅ 2 yrs), the following results were obtained:

$$p_o = e^{-(5 \cdot 10^{-3} + 10^{-4} \cdot 2)^4 \cdot 10^8} \quad (5)$$

which gives: «p_o» = .93. The value of «p_o» decreased to about .35 for a radiation dosage about twice the one considered for exposed women and a latency time of a single yr. This frequency was artificially set in good agreement with Literature."

4. Forfemale **breast cancer induction 2** different hypotheses can be made: a) the same difficulty of each step, but *more steps; b)* the same number of steps, but of a *more difficult kind.* For seek of simplicity only hypothesis (a) was considered. For «p» (frequency of mammary tumours in women) the value was referred to an X-rays dosage in the order of the hundreds of rads,[1] similar to the experiment considerated for the rats. In this case, making reference to equation[4] one had:

$$Q8 = e^{-(5 \cdot 10^{-3} + 10^{-4} \cdot 24)^x \cdot 10^{11}} \quad (6)$$

where x is the unknown exponent that has to be known. After few passages,

$$x = \frac{-29.2}{-4.9} = 5.96 \quad (7)$$

The interesting conclusion seems to be that, in case of a breast cancer, women have a latency so much longer than rats, because the steps involved in the carcinogenic process seem to be *one and a half time the steps necessary for female rats:* for instance 6 against 4 (Fig. 5).

It is interesting to note that this ratio remains unchanged even assuming a different number of steps. If, for instance, a HGPRT type-mutation frequency is given to a single step, a ratio of roughly 3:2 will be obtained for women and rats respectively.

C) CONCLUSION

The major conclusions following this brief analysis about the biological effects of X-rays on DNA and cells, with special regard for mammary tissue, are:

1. Biological models tend to favour a **non linear relationship** between dosage and effect, especially when comparing very small and average cumulative dosages. However, to Authors' knowledge, no experimental evidence of this fact exists up to now.[17]

2. It is probably possible to reach, during the entire life of a human being, **cumulative dosages** of about 200 rads or more; therefore the cumulative radiation exposure during the lifespan, should be recorded for each patient.

3. Radiological examinations in young asymptomatic women should be viewed with even more caution, in terms of risk-benefit assessment, considering the essential role played by **latency time** in tumour development.

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Chromosomall damages during diagnostic ascertainments

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A) INTRODUCTION

The effects of ionizing radiation can be biochemical and structural, including transitory delay and inhibition of mitosis, cell death, chromosomal and chromatidal aberrations, metabolic and functional alterations.

1. The **damage** can be of 2 types: somatic or hereditary. The alterations are most evident in germinal cells of the epithelium and in blastocytes of the hemopoietic organs due to their high level of metabolism which impedes the repair mechanisms. Partial repair occurs at the moment the damage takes place, but repair is ,-,/not constant and decreases with increased doses and lengthened times of exposure. The defense mechanisms are different for the various tissues and also differ from organism to organism.

Since 1970 the chromosome aberration test has been considered a biological indicator of the effects of ionizing radiation. Since chromosome analysis is done essentially on peripheral blood lymphocytes, it must be kept in mind that a large quantity of these cells live for long periods and are often not involved in the proliferation cycle. Consequently, the chromosoma1 aberrations can be seen long after the exposure.

An increased incidence of leukemia has been documented with epidemiological studies in the survivors of Hiroshima and Nagasaki. Also patients suffering from anchylotic spondylitis, who have had their vertebral column irradiated, and radiologists have been reported as having an increased incidence of leukemia and an elevated number of chromosoma1 alterations.

Simple diagnostic procedures can be leukemogenic in certain cases. For example, children born to women who had been exposed to X-rays during pregnancy have a high probability of developing leukemia before the age of 1O.[9] No threshold value is known for radiation damage, therefore the effects of radiation at levels inferior to those set by LAW (5 rem/yrs to the critical organs) are not known.

2. Mammography (M.) is the most reliable method for breast cancer detection. Since X-rays are used in this exam, fears have been expressed regarding the safety of the procedure. The risks involved and the benefits obtained through the M. examination have yet to be established.

The physical examination (P.E.) is not sufficient for the diagnosis of a breast cancer, because a tumour may be non-palpable for a long period. Since the better survival rate depends on the earliest diagnosis only the M. examination is able to achieve it. From 1960 to the present time the skin dose has dropped from 8-12 rad to 0.2-0.3 rad per exam. Nevertheless the problem concerning risk remains, because not all Senologic Centers use the low-dose procedures.

3. Regarding the **risk factors** of the M. examination 2 considerations have to be kept in mind: a) the breast is *sensitive* to X-ray radiation. An increase in breast cancer has been reported in women subjected to notable doses of radiation such as radioactive fall-out, repeated fluoroscopy and radiation treatment for mammary abscesses. The tumoural induction seems to have a latency of approximately 10 yrs. According to recent studies, 1 rad/ 1.000.000 women would theoretically produce 6 breast cancers/yr for the lifetime of the woman, after a latency of 6-10 yrs. b) The risk is *directly proportional* to the dose and the latency, on the contrary is indirectly related to the dose.

B) CHROMOSOMAL DAMAGES INDUCED BY MAMMOGRAPHY

Possible chromosomal damages induced by M. examination were studied at the I.S.T. according with that previously performed in workers exposed to small and continuous expositions. Sixteen women aged from 27 to 72 yrs were chosen from the patients who are seen at the Breast Screening Center of I.S.T. The same M-unit was used and all women were exposed to the same dose, approximately 1 radl exam. Blood samples, as control, were taken immediately before the M. examination. A second sample was taken 10 days later and a third after one month. The women chosen had not taken any drugs that could have interfered with the metabolism of hematic cells or cell division in the six-month period preceding the exam. Patients having tumours, diseases related to congenital chromosomic irregularities, recent viral infections and disorders of the hemopoietic system excluded from the study group. One hundred cells in metaphase were counted for each patient. The method used was that of MOORHEAT (1960). Chromosome evaluation was based on numerical and morphological variations: chromatid breakage, chromosome breakage, dicentrics and rings.

C) RESULTS

Examination of the cells in metaphase and the resulting chromosome maps have shown the presence of a series of chromosomal alterations in all three blood samples.

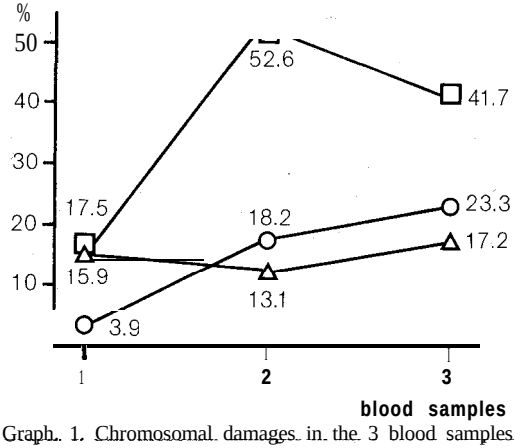
1. First blood sample (Tab. I). Out of a total of 1563 cells observed in metaphase, 236 (15%) numerical variations were seen, of which 15 (0.9%) were polypoid, 156 (9%) were the result of chromatid breakage and 33 (2.1%) were due to chromosome breakage. One case (0.06%) of endo-duplication was observed. An interesting comparison can be made between the results of the first sample and the results observed in workers who have not been exposed to a radiological risk. The data obtained in this study showed a higher level of chromatid irregularities and presented chromosomal anomalies not observed in the above-mentioned subjects. This suggests the possibility that the samples already had inherent risk characteristics.

2. Second blood sample. Out of1570 cells observed in metaphase, 301 (19.1%) numerical variations were noted, of which 29 (1.8%) were

Tab. I. **Chromosomal damages in the blood samples. 1 = before mammography; 2 = ten days** and **3 = one month after mammography.**

Anomalies	1	2	3
Numerical variations	15.9%	19.1%	17.2%
Polypoid	0.9%	I .8%	1 .5%
Chromatid anomalies	9.9%	26.3%	41.7%
Chromosomal anomalies	21.%	10.1%	23.3%
Duplication	0.06%	0.06%	
Fragment		0.2%	0.1 7%
Ring		0.06%	
Dicentric		0.06%	

polypoid, 413 (26.3%) due to chromatid breakage, 160 (10.1%) the result of chromosome breakage. Three fragments were seen in one case (0.2%), a sample with «minutes» in another (0.06%) one case with endo-duplication (0.06%), one ring (0.06%), and a dicentric (0.06%). Comparing the second sample taken with the data of the workers exposed to small continuous doses of radiation, an elevated number of numerical anomalies both chromosomal and chromatidal was demonstrated. It should be noticed that the percentage of dicentries (0.063%) in this study is inferior to that reported for workers exposed for 11 to 15 yrs, but in the last group rings were observed.



Graph. 1. Chromosomal damages in the 3 blood samples (△ = numerical variations; □ = chromatid and ○ = chromosomal anomalies).

3. Third blood sample was possible in 6 patients only (Tab. I). Out of 573 cells in metaphase observed, 99 (17.2%) numerical variations were counted, of which 9 (1.5%) were polypoid, 239 (41.7%) were due to chromatid breakage, 134 (23.3%) were the result of chromosomal breakage. A fragment was seen in one case (0.17%). Remembering that the third blood sample was taken in only 6 women, the Graph 1 explains the results of the 3 samples in the same women. Totally, the numerical variations and chromosomal anomalies had a slight raise while the chromatid anomalies had a decrease. Referring to each woman, a chromatid and chromosomal anomalies decrease was observed in 3/6 women, and only chromatid anomalies decrease in l/6. It must be emphasized that, even in the cases where the anomalies diminuished, a return to the situation seen in the first sample was not observed.

4. Statistical elaboration gave $p < 0.05$ significance when a comparison was made between the first and second samples and between the first and third. The difference between the second and third samples was not statistically significant.

D) DISCUSSION

Comparison of the data of the first and second samples revealed that the numerical variations, polypoides, chromatid and chromosome anomalies increased.

In the 6 cases in which the 3 samples could be taken, it was revealed (Graph 1) that numerical anomalies and polypoides fluctuated. The importance of the variations is relative since they are influenced by several endogenous and exogenous environmental factors.

The chromatid breakage diminuish from the second to the third sample. Finally the chromosomal anomalies were increasing from the first to third sample. 3/6 women present an increase and the other 3/6 women present a decrease with respect to the second sample. It can be hypothesized that the time (1 month) between the M. examination and the third blood sample was not sufficient for chromosome repair individual factors had too great an effect on the results.

A self criticism must be immediately made regarding the population studied. The number of patients was small and the subjects were not homogenous in terms of age and risk factors that could influence the results. Sampling and selection of the study population should be improved, obtaining an asymptomatic control group of adequate number containing women with no risk factors. However, the damage induced in human chromosomes by M. examination is evident from the data collected. Consequently, the M. examination confirms itself as the most important method in breast cancer diagnosis, but it must be reserved to symptomatic patients or high risk groups. In this way the risk-benefit ratio is still advantageous. In asymptomatic women and in women requiring repeated check-ups, the non invasive techniques are suggested, such as C.T. examination, according to its low costs, its safety and its good diagnostic accuracy when used in association with physical exam.