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ENDOCRINE ASPECTS OF CANCER: AN EPIDEMIOLOGICAL APPROACH

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Summary—Over the last 2 decades epidemiologists have increasingly used parameters of endocrine function in their studies. Prospective cohort studies offer methodological advantages in view of the latency between the relevant hormonal exposure and the clinical onset of cancer. Examples from the author's experience are provided. Evidence is mounted for an important "time window" for breast cancer development between menarche and the birth of a first child.

Hormones in their wider sense are so intimately related to all living matter that one must define the limitations of this subject straightaway. I shall attempt to describe how we try to get an understanding of the hormonal aspects in the aetiology of cancer in humans other than by extrapolating results from animal experiments.

Animal models can be useful in gaining an insight into mechanisms of oncogenesis but I hold the view that such insight relates more to principles of carcinogenesis than to the kind of understanding which lends itself to preventive measures.

The approach of the epidemiologist to cancer (in fact to any disease), to describe first and then to base further analysis on the results of this description, seems to be a sound one. Very often it is necessary to be critical of so-called clinical impressions, e.g. surgeons may conclude on the basis of the age distribution of a particular kind of cancer that it is most frequent at age 50. However, as soon as the population at risk is taken into account the risk may be shown to increase steadily with age. In such a way loose statements about the influence of menopause can be modified.

Another premature conclusion which one hears fairly often is the interpretation of any differences in disease frequency, between men and women, in hormonal terms. The sexes, however, differ in many ways from each other, in occupation and lifestyle, and such factors have to be considered carefully before jumping to conclusions of an endocrine nature.

The epidemiological description sooner or later leads to the formulation of aetiologic hypotheses in which not only statistical facts but also the results of animal experiments and clinical observations are integrated. The kind of studies involving human beings is of an observational rather than of an experimental nature in view of ethical considerations. The epidemiologist is (or at least, should be) well aware of the dangers of this approach, he runs the risk of overlooking hidden selection biases or factors which tend to confound any relationships which he is looking for.

In interpreting relationships between factors and events time is paramount. Thus, investigations have to be made either in a retrospective or in a prospective way. Studies of the former type are usually called case–control studies and those of the latter type, cohort studies.

In case–control studies a series of cancer patients are compared with a control group, concerning variables which for instance relate to past experience of hormonal relevance. The interpretation of any differences found can be difficult and even misleading, e.g. cancer of the uterine cervix; patients with this disease tend to have more children than controls, however this reflects sociocultural differences rather than hormonal exposure. On the other hand, patients with cancer of the breast tend to have fewer children than controls. This association has been explained mainly by the fact that an early full-term pregnancy protects against breast cancer and this may be explained in terms of hormones, notably by the pregnancy-induced differentiation of mammary glands.

Whether or not to interpret any statistical relationships concerning hormonal action,

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could be a point of departure for studies in experimental animals. This type of interplay between experimental medicine and epidemiology seems to be more conducive to medical progress than the sort of autonomous growth of cancer research in rodents, which we have seen over the past decades.

In cohort studies large groups of human beings are characterized according to their exposure to risk factors, in terms of lifestyle and other factors, e.g. endocrine variables like pregnancies, menstrual abnormalities and contraceptive or other pills. They are then followed up for many years concerning their disease experience. Needless to say this is a costly affair and one should not undertake such endeavours unless there are attractive hypotheses to test.

It was a clever idea of Bulbrook *et al.* [1] to add a new element to the prospective study methodology, viz. to collect specimens of urine from all participants at the time of entry to the study, which was carried out on the island of Guernsey. Thousands of specimens were frozen and stored for years until the onset of breast cancer in a number of women. Unfortunately, their hypothesis about the effect of androgens as reflected by their metabolites did not work out. Nor could our group at Utrecht demonstrate such an effect in a cohort of somewhat older women [2].

However, the advantage of collecting biological samples in the framework of a prospective study over the case–control design is clear. I remember having read a proposal by the Dorfman group in the sixties which planned to measure various steroids in blood of breast cancer cases vs controls. Apparently the enthusiasm of new technology made them somewhat deaf to the weakness in design which implicitly assumed that a specific steroidal pattern existed in the patients long after the initiation of the cancer, viz. at the time of diagnosis.

Meanwhile we have become acquainted with the concept of carcinogenesis as a series of steps over a number of years. Therefore specimens of blood or urine or tumour tissue collected at the time of diagnosis are better suited to the study of prognosis than of causation.

Where do we stand today? Did we achieve anything tangible in our understanding of the role of hormones in the aetiology of human cancer? The answer is partly yes and partly no, depending on the complexity of the cancer concerned.

It is my firm opinion that the aetiology of endometrial cancer has been clarified. If anyone opposes this view in terms of our lack of knowledge at the molecular level I would reply that this kind of lack of detailed knowledge also holds for tuberculosis or measles which we nevertheless can fight effectively on the basis of scientific insight.

The various aspects of the aetiology of cancer of the endometrium, its geographic occurrence, the epidemic in California, its age distribution with preponderance at postmenopausal age, the association with obesity, the coincidence with certain ovarian tumours and the Stein–Leventhal syndrome and the relation with oestrogenic drug treatment can all be accommodated in one unifying hypothesis, that of unopposed oestrogenic stimuli.

We were actively engaged in this field in the sixties when we asked ourselves the question, if oestrogens are involved in the genesis of this cancer, could it be that the clinical syndrome of obesity, diabetes and hypertension so often seen in these patients is associated with postmenopausal oestrogen production?

We employed a technique from exfoliative cytology viz. the study of urinary sediments after staining with a modification of Papanicolaous method. This technique which produces pictures very similar to vaginal smears has the great advantage of enabling one to investigate populations and not only patients. By the year 1965, it had become clear to us that obesity was responsible for oestrogenic smears after menopause [3]. We secured evidence that there was a dose–response relationship, viz. the fatter the women the higher the likelihood of an oestrogenic smear [4]. Moreover, we showed that the oestrogenic response tended to be a continuous one and that it was of extra-ovarian origin [5].

In those days we thought that the oestrogens were derived from the adrenal cortex. Soon we learned from work by the MacDonald–Siiteri group that adipose tissue itself was the source of extra-ovarian oestrogens: a more causal explanation of our cytological findings could not have been imagined. In subsequent studies Poortman and Thijssen [6] confirmed the conversion of adrenocortical androstenedione into oestrone in postmenopausal women.

During the past decade several case–control studies have found that the production and excretion of oestrogens by women with endometrial cancer is higher than among controls.

Any conflicting results can be located in terms of the prevalence of obesity in the control group. Some authors considered it necessary to correct for body weight in their case–control comparisons. However, if differences in oestrogen levels seem to disappear after controlling for body weight, this does not invalidate the oestrogen-cancer hypothesis. The findings agree with the concept that continuous oestrogenic stimuli because of obesity in postmenopausal women increase the risk of developing endometrial cancer.

Scientific efforts are a promise but not a guarantee of success. Many endocrinologists have devoted their best years to trying to understand breast cancer. One of them, Mick Bulbrook once remarked that all of us may have studied the wrong hormones with inadequate methods at the inappropriate time in the evolution of this type of cancer.

The breast cancer problem is pressing because of its severity in terms of both its incidence and its prognosis. Large-scale screening with mammography may be helpful but after all, this is a sort of stop-gap which should not lead to fewer efforts to understand the aetiology of the disease.

Epidemiological studies have led to the widespread belief that breast cancer in one way or another is related to Western-style nutrition. In the U.S.A., coordinated efforts have been made to develop hypotheses in this domain. Dietary fat is one candidate, but up to now no convincing evidence has been accumulated. Neither is there any insight into how this kind of overnutrition would affect the endocrine system which undoubtedly is involved in the chain of events.

Obesity has been proposed by us in the sixties because of its analogy with endometrial cancer [7]. Some evidence for this has been found by us and several other groups but the issue is complicated by the fact that body height seems to be a risk factor too. Thus, in terms of a weight-for-height factor like Quetelet's index the differences are small or absent. Nevertheless, obesity has been found by many authors to worsen prognosis, which suggests that it may play a role in the late stages of the cancerous process [8].

About 15 years ago Sherman and Korenman suggested that there are 2 periods in a woman's life in which anovulation or luteal insufficiency creates a "window" of relatively unopposed ovarian oestrogenic stimulation to the breast.

The first period was thought to occur in the postmenarcheal years and the second during the climacteric period before menopause. I shall touch upon the issue of a "window" at a young age later and firstly address the hypothesis of a possible breast cancer risk of luteal insufficiency in the premenopausal years. In Paris, Mauvais-Jarvis in particular defended this idea and recommended preventive treatment with progestagens.

It appeared to us that the best way to tackle this issue was to design a prospective study. A large cohort was assembled of 12,000 women between the ages of 40 and 50 who were willing to keep a menstrual calendar for 4 months and collect a 12 h specimen of urine on day 22 of 3 consecutive cycles. These samples were frozen at $-20°C$ [10].

When a case of breast cancer was reported to the cancer registry the respective urine sample was assayed for pregnanediol together with samples of age-matched controls. In the statistical analysis we employed a sequential graphical technique for the case–control comparisons which enabled us to conclude, after analysing some 45 pairs of women, that those who were to develop breast cancer had normal pregnanediol excretion during the years before clinical diagnosis.

Over the years a number of facts have induced me to believe that we should re-orient our studies in the aetiology of human breast cancer towards much younger age groups, notably to girls at puberty and adolescence. There are several compelling arguments for such an opinion:

1. Trends over time in breast cancer incidence, notably in Iceland (where records have been available since 1911) have made it clear that the rise from a low to a high incidence during the 20th century took place cohort-wise, viz. each successive birth cohort got a higher incidence than the previous one [11]. Thus, a decisive step in the genesis of breast cancer must occur early in life.
2. The experience of Japanese migrants to the U.S.A. (Hawaii, CA) tells us that those who were either young at migration or were born in the U.S.A. had a much higher incidence than those who migrated after having reached adulthood [12] (whose incidence remained as low as that prevailing in Japan).
3. In a number of case–control and cohort studies it has been found that a tall body

height increases breast cancer risk [13]. This points to influences of Western-type nutritional abundance which affects the female breast at the relatively young age when body height is being determined. It is being noted in passing that the young migrant Japanese grew 10 cm taller in the U.S.A. than their parents or grandparents in Japan.

4. In several case–control studies it was found that breast cancer is associated with a relatively early age at menarche, the first menstruation in a woman's life [14]. Circumstantial evidence suggests that, age at menarche depends on nutritional status. Frisch *et al.* [15] believes that a critical body mass is needed for the beginning of menstrual periods in a woman's life. Slender somatotype at a young age (college athletes) is associated with a lower breast cancer incidence later in life.

In a multi-national study of oestrogen excretion MacMahon *et al.* [16] showed that oestrogen levels not only depend on age but also on age at menarche, i.e. at equal calendar ages girls with an early menarche have higher oestrogen excretion (and probably, oestrogen production) than those with a late menarche.

Therefore, nutritional factors seem to determine not only the duration of menstrual life but also the oestrogen levels during puberty and adolescence. If this finding can be confirmed it would act as a mediator between Western-type nutrition and proliferative stimuli of breast tissue before the first pregnancy occurs. From radiation biology we now know that the proliferating breast at that age is particularly vulnerable to carcinogenic "hits".

It has also been shown by MacMahon *et al.* [17] that early full-term pregnancies confer a degree of protection against breast cancer. It is highly likely (and experimentally demonstrated) that the differentiation of breast epithelial cells as a result of the action of the hormones of pregnancy renders those cells less vulnerable to the effect of carcinogens [18]. Therefore, women in Western societies undergo the joint effect of 2 kinds of factors, (1) early breast development because of rich nutrition and (2) late differentiation of breast cells because of deferred motherhood.

It appears to me that this hypothesis could be a useful starting point for both epidemiologists and endocrinologists in a renewed attempt to understand the aetiology of human breast cancer. It will not be easy to provide direct evidence for a relationship between nutrition and hormones at a young age and the occurrence of breast cancer some 30–40 years later. The puzzle will be of a complex type with due recognition of intermediate stages in the carcinogenic process. It would be of tremendous value if new non-invasive techniques were available to study the female breast. The study of cancer requires progress in the medical sciences on a broad scale.

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