Technical Papers

Thiamine Deficiency and High Estrogen Findings in Uterine Cancer and in Menorrhagia[1]

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While studying vaginal and cervical cytology smears for a diagnosis of uterine cancer, the observation was made that over two-thirds of the 150 cases proven to be cancer showed evidence of abnormally high endogenous estrogenic activity. This observation assumed a greater significance when postmenopausal cases of 60 and 70 years of age manifested a cytology picture of estrogenic cornification similar to that of a young woman in the regenerative phase of the sex cycle.

The quantitative production, metabolism, and excretion of the estrogens is even today obscure. Blood assays have proven unsatisfactory, and urinary levels are a measure of the excretion but not of the endogenous level. Our studies lead us to believe that perhaps the simplest and most sensitive measure of endogenous estrin at our disposal today is the specific hormonal cornification reaction manifested in the cells of the squamous epithelium of the vagina.

In humans and in monkeys the estrogens manifest their presence in the vaginal mucosa by a cornification change in the cells, first described by Allen (*1*), which is accompanied by proliferation of the vaginal and cervical squamous epithelium. This growth change is related to the deposition of glycogen in the squamous cell, which is mediated and controlled by the force of the estrogenic stimulus. The vaginal epithelium of the average postmenopausal female is made up largely of basal cells which contain no glycogen, and cornification is absent. Under the influence of the estrogenic hormone the deposition of glycogen may be brought about, and cornification of the squamous cells occurs whether the subject be postclimacteric or following oöphorectomy. This was demonstrated in monkeys by Robertson, Maddux, and Allen (*17*), and in humans by Krumm (*13*). We have confirmed the latter's observations in our own laboratory. We have found further confirmation of the estrogenic activity by studying the endometrium of senile patients suffering from cancer of the cervix. Many of those showing cornification in the smears also exhibited endometrium identical to that found in an active regenerative phase, while in other cases the estrogenic stimulus was sufficient to produce a picture of glandular hyperplasia.

The findings of various investigators using laboratory animals would appear to indicate that liver damage may be induced by dietary deficiency which results in interference with estrogen inactivation. Biskind and Biskind (*3*) demonstrated that in female rats the liver loses its ability to inactivate estrogen in vitamin B-complex deficiency. Addition of brewer's yeast to the diet was found to restore the inactivating mechanism (*5*). The amount of estrogen inactivated by the liver could be controlled at will by withholding the vitamin B complex or by restoring it to the diet.

Singher and his associates (*18*) have demonstrated that thiamine and riboflavin are essential in the metabolism of estradiol by liver slices. The inactivation of estradiol is dependent upon the concentration of these vitamins in the liver, and they state that it seems possible that these vitamins may be involved in estrogen metabolism through their role as members of an oxidative enzyme system.

It has been recognized that menorrhagia and metrorrhagia may occur early in the course of cirrhosis of the liver (*12*), and the work of György and Goldblatt (*9*) and of others has demonstrated that cirrhosis of the liver is known to result from nutritional deficiency. Sources of the B complex have been shown to protect the liver against a variety of toxic agents such as lead, arsenic, and dimethylaniloazobenzene, which cause functional and morphologic damage to this organ. In addition to this evidence, Goldberger (*8*) has shown that menorrhagia may occur in pellagra.

Pincus and Graubard (*15*), in studying estrogen metabolism in seven women suffering from cancer of the uterus, concluded that these cases metabolized the estrogen in an abnormal manner. Their most striking finding was that the total estrogen urinary output after estrone and progesterone administration showed only a slight or negligible increase. The fact that the estrogen could not be accounted for might be explained on the basis of a liver block.

In treating women suffering from menorrhagia, metrorrhagia, and other disorders with large doses of B complex and liver extract, the Biskinds (*4*) report

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correction of the functional disorders in a high percentage of cases.

For the past year we have studied 180 ambulatory gynecologic cases suffering from menorrhagia or amenorrhoea. While these patients were not accessible for biochemical assays, it was found possible to add to the clinical findings the simple estrogen determination in the cytology smears before and after administration of thiamine chloride and the other constituents of the B complex and liver extract. While these studies have not been published or completed as yet, one salient feature has been noted, viz., that some definite relationship exists between the intake of B complex and the rise and fall of the estrogenic levels in the body as manifested by the cornification counts in the vaginal smear. Clinically, uniform improvement has been shown in cases other than those proven to have an inflammatory etiology, and the cytology smear findings have given us additional laboratory evidence to help explain why improvement occurred. In some cases we have found a dramatic drop in the cornification level after administration of B complex, as if some part of this preparation were acting specifically in the elimination of the estrogen. It is regrettable that urinary estrogenic levels were not followed at the same time. One might expect that if a block were actually present in the liver, the blood levels and the vaginal cornification would be high and the urine levels low. Coinciding with a drop in the cornification level subsequent to B-complex administration, the urinary estrogen should increase. (Further cases are being studied with a view to verifying this.) Also, the evidence would be more complete if liver function tests were available to assess damage such as might interfere with elimination of the estrogens.

Whether or not the accumulation of uncertain quantities of various estrogens in the body would act as a carcinogen to the susceptible Mullerian tissues and the glands of the breast is open to question. It is known that estrogen is essentially a growth-promoting hormone acting most specifically on the endometrium, which, during each monthly cycle, undergoes the most rapid growth occurring physiologically in the body. Its effect is also specifically exerted upon the squamous epithelium of the vagina. But this same type of epithelium covers the cervix, shows the same cyclic growth changes, and is the tissue most commonly involved in malignancy in the human female.

The role of the estrogens in inducing proliferation of the vaginal epithelium was described in castrate monkeys by Allen (*2*) and has since been confirmed in humans by Shorr and others (*6*). As a result of the injection of estrogens an enormous growth in the vaginal epithelium can be induced, associated with an increase in the number of growing bulbs along the basement membrane, the cells of which contain many mitotic figures. Allen has cautioned that the increased incidence of malignant change following administration of estrogens to cancer-susceptible strains of animals must serve as a warning to the potentialities in the human. While the short life-cycle of mice permits an early recognition of what is carcinogenic, the same influence acting upon human tissues during the same fraction of a lifetime might produce a malignant growth in man. Therefore, it seems possible that errors of diet leading to persistent or intermittent deficiency of such elements as thiamine might cause a persistent estrogenic growth stimulation over a period of years which, acting upon a "locus minoris resistentiae" such as an erosion, ultimately produces a malignant neoplasm.

From our own experiences we may recall cases where we must incriminate an estrogen in the development of cancer. The development of carcinoma in the breast following weekly injections of an estrogen for menopausal therapy is in itself inconclusive evidence. But when such cases occur time and again, they assume greater significance. Dr. J. S. Henry (*10*) has reported two cases in whom the administration of stilbestrol over a period of many months was followed by the finding of malignant growths in the uterus.

It has been found that the implantation of ovarian tissues into mice produced cancer of the breast in cancer-susceptible animals, and it was thought from this that some secretion of the ovary played a role in the production of cancer. It has long been recognized that certain coal tar products produce cancer with greater certainty than any other known chemical or physical irritant. The chemical structure of the responsible substance has been found to be similar to that of some of the active substances in the sex hormones.

Rhoads (*16*) has presented evidence to show that in rats the administration of one of these two-benzene-ring substances, "butter yellow," will result in the development of cancer when their diet consisted of polished rice and carrots. If liver or yeast was added to the basal diet, no cancer occurred. Here, clearly, was an experiment in which a dietary constituent, rich in its content of the vitamins of the B complex, was protective against induced cancer.

A recent survey of evidence of estrogenic activity in 62 granulosa cell tumors has been made by Hodgson, Dockerty, and Mussey (*11*). They stated that "evidence of hyperestrinism is afforded in our series by symptoms of precocious puberty, amenorrhoea, and post menopausal bleeding." They reported proliferative endometrium in 67 per cent of their cases.

In 38 postmenopausal patients who had granulosa cell tumor they reported 21 per cent as showing an independent endometrial carcinoma, and in one-third of these cases carcinoma of the breast with axillary metastasis also developed. They concluded that "this phenomenon of co-existent ovarian, endometrial and mammary carcinoma in the human being bears a marked similarity to the results of experiments on laboratory animals in which estrogen stimulation appears to be a factor in carcinogenesis."

During the course of making routine cytological studies on some of our gynecologic bleeders, three cases were encountered in whom similar cytology findings prompted us to investigate the nutritional biochemical status of these patients.

The three cases were 14, 29, and 64 years of age, respectively. The first was suffering from pubertal bleeding which followed a total dental extraction. The other two showed evidence of malignancy in cytology smears, as described so expertly by Papanicolaou and Traut (*14*). All were bleeding excessively, and all showed abnormally high estrogen levels in the cytology smears as well as thiamine deficiency when their urinary excretion levels were analyzed by Dr. Andreae, of the Nutrition Department of the McGill University Clinic.

In the present investigation the vitamin status was estimated by a vitamin-tolerance technique. Following a vitamin-free supper the urine from 12:00 midnight to 8:00 A.M. was collected in a bottle containing acetic acid. In the morning, after a vitamin-free breakfast, an intramuscular injection of 1 mg. thiamine and 1 mg. riboflavin was given and the urine collected for the subsequent four-hour period. Both specimens were analyzed—by the thiochrome method of Wang and Harris (1943) (*19*) for thiamine, and by the Ferrebee (*7*) method (1940) for riboflavin. Patients with below-normal excretion values were classed as deficient in thiamine and/or riboflavin.

The other vitamin B factors measured were normal in amount. Liver function tests were taken on all cases with uncertain results. The first case showed a prolonged prothrombin time, and the second an abnormal cephalin flocculation test (plus 2 in 24 and 48 hours), while the third showed a bromsulfalein retention of 10 per cent. Dr. M. Hoffman, who has studied liver function tests intensively, states that no single test has as yet been elaborated to assess liver damage, and it is possible to have liver damage sufficient to interfere with estrogen elimination without this damage being detectable by the tests at present at our disposal.

The endometrial patterns in these cases verified the high estrogen levels shown by cytology counts. In the first two cases varying degrees of active hyperplasia were found. An endometrial biopsy was not obtained in the third case.

The most striking feature, however, was the evidence of growth manifestations in the 29-year-old case, who exhibited most unusual evidence of proliferation in the squamous tissues of the cervix. Cytology smears taken from the cervix and vagina showed many cornified cells, but in addition there were many immature and anaplastic squamous cells with huge nuclei showing numerous chromatin bundles. Atypical cells were found showing all grades of variability from the normal basal cell to the most bizarre malignant type of cell with the nuclear heteroplasia characteristic of cancer. These findings appear the more remarkable in that no cancer was visible clinically—only a suspicious-appearing erosion encircling the external cervical os. Following surgery, the lesion from which these cells had become desquamated was located. Pathological study left an element of question, however, when one pathologist diagnosed a squamous intraepithelial carcinoma, while another diagnosed it as "precancerous secondary hyperplasia" of the cervix.

The tissue study in the case of the 14-year-old case of pubertal bleeding proved interesting also. Grossly, the tissue was thick, abundant, and polypoidal, while microscopically, various areas presented an appearance of overstimulated proliferative glands with mitosis and adenomatous formation such as would make the pathologist think twice before eliminating a diagnosis of precancerous change if found in a 40-year-old. Yet such a diagnosis would seem fantastic in a child of 14 years!

The third case exhibited a typical squamous carcinoma of the cervix, an early small lesion, but a rapidly growing undifferentiated neoplasm.

One might readily expect that a cancerous victim in the throes of cachexia might show justification for deficiency, but investigation into the dietary intake reveals that in the two cases of neoplasm, the lesions were early and asymptomatic except for the bleeding. The 29-year-old case was suffering from chronic constipation, abdominal cramps, and nausea brought on by solid food, which restricted her to a liquid or soft diet. In addition, she was a moderately heavy drinker.

The other case revealed excellent nutrition externally, but she admitted that she dieted constantly to keep her weight down, and analysis of her diet revealed an inadequate thiamine intake.

Nutritional Studies

It perhaps seems remarkable in these days of scientific enlightenment and dietary refinement that deficiency would occur irrespective of the economic status of the patient. It has long been a general

feeling among gynecologists that dietetics were not directly concerned with the production of pelvic pathology, and that the need for vitamins in particular was generally overstressed. It would seem logical that the average person with a normal appetite for the various staple foods should not develop a deficiency due to an inadequate intake. A study of thiamine physiology and metabolism reveals evidence to indicate that this substance would appear to be particularly vulnerable to intermittent or chronic depletion without gross deficiency in the diet as a whole. This tendency would appear to depend upon the fact that little thiamine is stored in the organism and the amount is sufficient only to maintain proper life for a few days. A daily intake of thiamine is necessary, and the organism absorbs only enough for the immediate needs, the excess being destroyed or excreted. More is required when alcohol is imbibed or when a high carbohydrate diet is taken. Fats, on the other hand, spare thiamine. This may account for the finding of deficient levels in well-nourished but obese patients who are constantly dieting. We have found that a vicious cycle may occur, as anorexia and constipation frequently develop in the presence of a deficiency. Therefore, the greater the anorexia the more pronounced the deficiency.

With so much discussion of vitamin therapy nowadays, one might be led to believe that they are a "cure-all." The truth, however, is that vitamins are only nutritional elements, and to say that a vitamin would cure a deficiency or a disease resulting from a deficiency is simply to say that a well-balanced diet, properly absorbed and utilized, would have prevented the disorder.

After observing the consistency of finding "B" deficiency in so many gynecologic cases, one naturally wonders if half the population may be deficient. If so, the reported findings would lose much of their significance. But this is not the case. Dr. Andreae reports that of the cases in the Royal Victoria Hospital tested because of a suspected deficiency, only one in eight has been proven to be deficient. Further, our own controls of nongynecologic cases reflect the same normalcy.

Speculation arising from an analysis of the findings in the cases herein presented suggests the inference that the thiamine deficiency was causally related to the pathological conditions found. A possible mechanism to explain functional bleeding and the development of a malignant growth in the estrogen-susceptible pelvic tissues presents itself. Is it possible that the dietary deficiency, acute in the case of pubertal bleeding, and chronic in the two cases of cancer, produced some barely demonstrable liver damage which was sufficient to inhibit this organ from conjugating the estrogens necessary to permit their inactivation? Is it possible that the accumulation of the estrogenic substances produced the overgrowth of the endometrium, resulting in endometrial hyperplasia with its characteristic bleeding? And is it possible that as a result of such liver damage the estrogen accumulating in the system, unable to escape, continually exerting its growth stimulation upon the pelvic tissues over a period of years, leads to an actual carcinoma?

Summary

The finding of abnormal estrogenic activity coupled with thiamine deficiency in cases of menorrhagia and uterine cancer suggests a possible etiological correlation between the dietary deficiency, the abnormal estrogen level, and the pathological lesion.

The specific element deficient in these cases was thiamine, while the other B factors were normal.

Preliminary report of the evidence is made in this small series while more extensive studies on a large series of cases are being pursued.

Cornification in cytology smears was used to study estrogenic activity, since the present study was prompted by cytological findings; the method is simple, practical, and reasonably accurate. The urinary estimation measures only the amount excreted, and if liver impairment actually is present, the quantity excreted would not give a true index of the amount retained in the body.

Further studies are being undertaken in which estrogenic, urinary, and cornification levels are being compared before and after thiamine administration in cases proven to be deficient.

Supplement. Since the original submission of this paper, further cases have been investigated. Of 20 cases suffering from proven uterine cancer, 90 per cent of these were found to have a low thiamine excretion coupled with an abnormally high endogenous estrogen level. The average thiamine excretion level was found to be less than half the average levels found in a control group which showed normal estrogen levels.

Of the 20 cancer cases studied, 20 per cent showed also a deficient excretion of riboflavin.

A similar group of menorrhagic patients was studied and showed the same co-relation of thiamine deficiency and an abnormally high estrogenic level.

In an attempt to explain why some cases develop menorrhagia and others cancer, the time element may enter the picture. Since most people develop a fixed habit of diet, a dietary error over a period of time would tend to produce a chronic deficiency. If this acts on the human liver in the same way as in animals,

it would seem logical that even small amounts of estrin, not being inactivated by the liver would accumulate in the body and at the same time act as a chronic growth stimulant to the estrin-susceptible tissues, viz., the pelvic organs and possibly also the glands of the breast.

The fact that dietary errors tend to be chronic is shown in certain of our cancer cases, where even after cure, or a remission of months or years, they return to the same habits of diet and continue to show thiamine deficiency. While we must also consider the possibility of an internal metabolic defect leading to the deficiency, this would tend to be countered by the fact that with correction of dietary intake the deficiency was corrected in our cases. Whether or not these cases require an increased quantitative intake of these essentials to maintain a normal level remains to be investigated.

Conclusions

When we couple the findings with the background of what has been proven in animals, there appears to be excellent circumstantial evidence to suggest that the nutritional deficiency may have been a primary factor leading to the malignancy.

Whether this low thiamine excretion is due to an actual primary dietary deficiency or to some internal metabolic change is a subject for further investigation. In either case, if this hypothetical mechanism proves to be correct, it means that we are presented with the means not only of diagnosing uterine cancer by cytological findings but of actually detecting a potential cancer-producing mechanism even before the cancer develops! Two simple tests would be used: (1) a cervical cytology test, which would tell (a) if the person had uterine cancer, and (b) if not, whether the endogenous estrogen level was abnormally high, in which case the second test should be done; and (2) a test of the urinary thiamine level.

The finding of a combination of low thiamine and abnormally high estrogen could be recognized as a dangerous precancerous linkage. Recognition of this would permit correction and possible prevention of the cancer.

No single track of investigation yields a conclusive answer to the cancer enigma, but when we piece together the evidence of animal experimentation, cytopathological, hormonal, and nutritional findings, there seems to be evidence that progress in the right direction is being made.

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The Antibacterial Activity of Protamine Zinc Insulin[1]

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As part of a program of chemical and biological study of proteinaceous antibacterials (*3*), it seemed desirable to test the effect of protamine insulin combinations of the kind used routinely in the control of diabetes (*1*). The antibacterial property of the protamines, clupein (*5*), and salmine (*7*), had previously been reported. Protamine sulfate prepared from shad of the Sacramento River by one of us (S.W.F.) has also been found to possess comparable activity (*2*). The experiments recorded below indicate that protamine is able to act as an antibacterial, even though combined in a relatively insoluble form with insulin.

The tests were conducted on cultures of *Lactobacillus arabinosus, Staphylococcus aureus,* and *Escherichia coli.* Both the supernatant liquid of centrifuged protamine zinc insulin preparations and the whole suspension, with appropriate controls, were tested. All tests were run in solutions buffered by phosphate. The contents of the tubes were initially at pH 7.1–7.4. *Lactobacillus arabinosus* was cultured at 30° in the special Bacto-Peptone medium of McMahan and Snell (*6*) and in the synthetic medium of Kuiken, *et al.* (*4*). The other two organisms were cultured at 37° in the usual beef extract broth. The observations recorded in Table 1 were made at 24 hours from tube cultures. All results were confirmed by plate cultures read at 48 hours. These plate cultures were made from well-shaken suspensions after an initial growth in

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