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A Quantitative Determination of Eosinophil Leucocytes in Patients With Carcinoma of the Prostate: A Review of Endocrine Factors Associated with Carcinoma of the Prostate

Dale Morrell Atkins
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A QUANTITATIVE DETERMINATION OF EOSINOPHIL LEUCOCYTES IN PATIENTS WITH CARCINOMA OF THE PROSTATE

A Review of Endocrine Factors Associated with Carcinoma of the Prostate

by

Dale Morrell Atkins

B.A., University of Colorado, 1943
M.D., University of Colorado, 1945

A Thesis submitted to the Faculty of the Graduate School of the University of Colorado in partial fulfillment of the requirements for the Degree

Master of Science

Department of Surgery
Division of Urology

1953
This Thesis for the M.S. degree by Dale Morrell Atkins has been approved for the Department of Surgery Division of Urology by [Signature]

In patients that showed reactivation of their disease, the eosinophil leukocyte count average approximately one-third less than that of the patients that were controlled by anti-androgenic therapy. Individual counts in the two groups of patients were compared and a great degree of overlap was noted; non-reactivated patients having eosinophil leukocyte counts, in many instances that were in the range of the counts observed in patients undergoing relapse. Low values were also observed in a group of clinically deteriorating patients with actively growing malignancies other than carcinoma of the prostate.
Atkins, Dale Morrell (M.S., Urology)

A Quantitative Determination of Eosinophil Leucocytes in Patients with Carcinoma of the Prostate

A Review of Endocrine Factors Associated with Carcinoma of the Prostate

Thesis directed by Robert A. Huseby, M. D., Ph.D.

The problem in this investigation was to determine whether or not serial quantitative determinations of the circulating eosinophil leucocytes could be used as an index in following the clinical course of reactivated prostatic carcinoma.

In patients that showed reactivation of their disease, the eosinophil leucocyte count average approximately one-third less than that of the patients that were controlled by anti-androgenic therapy. However, when individual counts in the two groups of patients were compared a great degree of overlap was noted; non-reactivated patients having eosinophil leucocyte counts, in many instances that were in the range of the counts observed in patients undergoing relapse. Low values were also observed in a group of clinically deteriorating patients with actively growing malignancies other than carcinoma of the prostate.
Although acute adrenal hyperfunction results in a decrease in the number of circulating eosinophils, it is felt by most investigators at the present time that the absolute level of eosinophil leucocytes in the blood at any given time does not accurately reflect the state of adrenal cortical activity. If the suggestive depression of the eosinophil count in patients with reactivated carcinoma of the prostate is significant, it may indicate only an adrenal response to "non-specific" stress rather than being a specific measure of adrenal hyperactivity with resultant androgen stimulus to the tumor. From the results of this study it would seem that the eosinophil leucocyte count cannot be used in following the clinical course of re-activated prostatic carcinoma patients.

This abstract of about 250 words is approved as to form and content. I recommend its publication.

Instructor in Charge of Dissertation
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INTRODUCTION

Early in history man observed that the testis controlled the development and state of the secondary sexual organs. Farmers used this information in the production of capons. Castration of bulls and other animals was found to be useful. Certain Asiatics took advantage of the suppression of the secondary sexual characteristics and sexual drive by castrating prepuberal boys to supply eunuchs for their harems. 43

In summarizing the early history of the scientific study of the prostate and its relationship to the testes, White stated:

"John Hunter observed that in the mole the prostate gland in winter was 'hardly discernible, but in the spring becomes very large and filled with mucus'. Owen confirms this and adds that 'the prostate gland in the mole begins to increase in February and acquires an enormous size and conceals the urinary bladder"
toward the end of March. Owen found similar changes in the prostate of the hedge hog. Griffiths examined for himself the generative organs of the mole and the hedge hog in and out of the rutting season, and found the above observations to be correct. He made a number of sections of the gland in the quiescent state, and others during the period of sexual activity. During the former period he found it composed of a few tubules lined by flattened and small epithelial cells, undoubtedly functionless, as regards the production of any secretion, but possessing the power of reproducing glandular epithelial cells at the appropriate period. Precisely similar phenomena were observed in the seminiferous tubules of the testicle, and in both the changes were preceded by an increased vascularity of the whole tract, determining an increased growth of all the glands and parts concerned in generation.

"As to the effects of castration, Hunter again was the first to note that while the prostate of the perfect bull is soft and bulky, that of the castrated animal is small, flabby, tough and ligamentous."

This knowledge of a general biological principle was put to little scientific use in the treatment of disease in man until the end of the nineteenth century.
White, after noting a decrease in the size of the prostate of castrated dogs—first the glandular and then the muscular elements—when the dog was sacrificed, accumulated 111 cases of castration for hypertrophy of the human prostate. At that time no differentiation was made between cancer of the prostate and benign hypertrophy. The evaluation of the results became so controversial that it is not possible at the present time to interpret these with any accuracy.

Orchiectomy had been extensively practiced in the treatment of non-cancerous prostatism but Young first described its employment in the treatment of prostatic carcinoma. Young's experience was limited to two cases and the results were negative.

Strohm in 1935 reported that placental blood injected intramuscularly acted as a great boon to pale old men suffering with malignancy of the prostate gland. Counsellor and Munger, independently, reported their experiences in the treatment of cancer of the prostate with irradiation of the testicles. In 1941 research in prostatic cancer received great impetus from the discovery by Huggins and his associates, that remarkable regression occurred clinically in the primary neoplasm and its metastases, after castration or admin-
istration of estrogens. These findings were subsequently verified by others. Wide acceptance of the thesis that androgens must be removed or neutralized led Huggins and his associates, as well as others, to search for sources of androgens in organs other than the testes and for ways of removing or neutralizing them.

Since the adrenal glands produce some androgenically active compounds, many urologists believe that, or wonder if, reactivation of prostatic cancer after periods of control by castration and estrogen therapy may be due to an increased androgen production by the adrenals. As acute adrenal hyperfunction causes a fall in the number of circulating eosinophils, it seemed possible that an increase in adrenal function, if such be responsible for the reactivation of prostatic carcinoma after castration and estrogen therapy, might be reflected in the eosinophil count. The question raised is, can the eosinophil count be used as one of the indices in following the growth of reactivated prostatic cancer in much the same way as the level of the serum acid phosphatase is used in many cases; hence the purpose of this investigation. Before presenting the results of enumeration of circulating eosinophils in patients with prostatic cancer, I would like to review the endocrine factors that are found in association with carcinoma of the prostate.
A REVIEW OF ENDOCRINE FACTORS ASSOCIATED WITH CARCINOMA OF THE PROSTATE

Distribution and Incidence.—Cancer of the prostate is characteristically a disease of men over the age of 40 years; the incidence increasing with advancing age. Joeck reporting in 1940 found no more than 25 cases of carcinoma of the prostate in individuals less than 30 years of age in the world literature. The youngest case on record is that of a 6$\frac{1}{2}$ year old boy. In 1948 Creevy referred to the previous literature on carcinoma of the prostate. He stated that the recorded incidence varied between 14 and 46 percent of all men past the age of 50 years, that in the United States the estimated number of cases at any one time is between 3,000,000 and 8,000,000, and that the disease is responsible for five percent of all deaths in men past 50 years of age. The United States Public Health Service estimates that one man in seven past 50 years has the disease.

In 1946 Nesbit and Plumb presented a report on the follow-up of 795 patients treated prior to the endocrine therapy era. The ages of their patients at the time of initial diagnosis varied between thirty-three and eighty-nine years with an average of 67.6 years. In the analysis of 1000 cases reviewed by Bumpus the average age was 65 years; the youngest was 42 years. Kimbrough and Lewis, commenting on the rarity of cases under the age
of 40 years, recently reported two cases; one of the
patients being 18 years old and the other patient 20
years of age.

Although racial incidence does not necessarily indi­
cate that hormones are involved in prostatic carcinoma,
it does suggest that some constitutional or genetic
factor may be important in prostatic carcinogenesis, and
certainly such factors may have their influence via the
endocrine system. In Chinese people cancer of the pros­
tate is a rare tumor. In a series studied by Hu and
Ch'in there was only one case of cancer of the prostate
in 379 carcinomas in Chinese men. 27

Ravich 50,51 in a survey of 1407 cases requiring
prostatic surgery found the startling low percentage of
1.8 percent of cancer among 1275 Jewish patients as
against 20 percent of cancer among non-Jews. This is a
significant figure in light of Young's 67 statement that
20 percent of all men over 60 have prostatic disease
and of these 20 percent have cancer. The incidence in
Negroes is identical to that in the Caucasian races. 65

To my knowledge there is not a single case reported
in the literature of carcinoma of the prostate in a
eunuch or in a true eunuchoid. The criteria for the
eunuchoid state are the changes in the secondary sexual
organs, and not the fact that the testis are small. This is good evidence that carcinoma of the prostate does not develop except in the presence of an androgen-producing testis.

The total excretion of urinary 17-ketosteroids has been determined in cases with prostatic carcinoma and compared with that in normal males in a similar age group. The results indicated that the total 17-ketosteroid excretion in older men is less than in men between the ages of 20 and 40, but that the cases with carcinoma of the prostate do not differ from control cases of a similar age group. As the biochemically active urinary androgens decrease there is a progressive increase in the incidence of carcinoma of the prostate. It would seem then either that androgenic compounds exert their carcinogenic effects early in life or that a lesser degree of androgenic stimulation over a longer period of time is the important thing as far as the development of prostatic carcinoma is concerned.

**Effects of Castration.**—Approximately 80 percent of patients with carcinoma of the prostate show clinical improvement when treated by orchietomy. In the patients who respond to treatment there is a decrease in the amount of pain, shrinkage of the primary tumor, x-ray evidence
of healing of osseous metastases, a better nutritional state and a decrease of the serum acid phosphatase level.

Dean, Woodard and Twombly in an experimental observation on 27 cases of cancer of the prostate determined the precastration and postcastration levels of estrogen and 17-ketosteroid excretion. Since castration was not thought of as affecting the adrenals, they expected the estrogens to remain constant and the 17-ketosteroids to fall, thus bringing about a shift in the estrogen-androgen ratio toward a higher estrogen and lower androgen level. These theoretical changes were not borne out by experimental observations. The estrogens dropped in all but one of their patients to about one-half the precastration level. The 17-ketosteroids tended to remain the same or, as time went on, to rise slightly. Gonadotropins from the anterior pituitary showed a definite postcastration rise in eleven cases, while in none of the cases was there a fall in the urinary gonadotropic hormone postoperatively. Scott and Vermuelin, in a similar study, obtained comparable results.

Attempts to correlate histological findings in removed testicles with the clinical effect of this surgical procedure, in cases of carcinoma of the prostate, have been unsuccessful. Angrist and Khoury attempted to
estimate the degree of function of the testis by the morphological appearance of the seminiferous tubules, of the Sertoli cells and of the interstitial tissue. They found that neither hyperplasia nor atrophy of the above mentioned morphological constituents, singly or combined, bore any relationship to the presence or absence of clinical improvement following the removal of such testicles.

**The Effects of Estrogens.**-- The percentage of patients with carcinoma of the prostate showing temporary remission of cancer activity after treatment with estrogens alone is about 75 percent. Clinical evidence of response to therapy is the same as in patients treated by orchiectomy. Occasionally, continued administration produces pain in the breasts and testis.

Dean and associates in a study similar to the one they did on the precastration and postcastration levels of estrogen, gonadotropin and 17-ketosteroid excretion, obtained assays on nine men before and after treatment with two to five mg. of diethylstilbestrol daily. The estrogenic assays on these men averaged 16.6 mouse units per twenty-four hours before treatment. After treatment this figure rose enormously because a certain amount of
the diethylstilbestrol was excreted in the urine. The 17-ketosteroids before treatment averaged 8.9 mg. of androsterone equivalent per twenty-four hours, but after treatment these metabolites fell in every case with the average being 5.4 androsterone equivalent. In a group of six patients studied for gonadotropic hormone excretion rate before and after stilbestrol, there was no post-treatment increase. Three showed no measurable excretion either before or after stilbestrol, while the other three, showing small quantities of hormone in the urine before the administration of the drug, excreted too little to measure after it was started.

From the point of view of hormones excreted in the urine, therefore, the treatment of prostatic carcinoma by orchiectomy differs considerably from treatment with stilbestrol. Castration seems to cut estrogenic excretion in half, tends to raise 17-ketosteroid excretion, and to release the pituitary from testicular inhibition so that it pours out its gonadotropic hormone in excessive quantities. Diethylstilbestrol raises the estrogenic excretion and decreases both the 17-ketosteroid excretion rate and the quantity of gonadotropic hormone in the urine.
Clarification of the true mechanism by which prostatic cancer regresses after castration or the administration of stilbestrol must await more detailed analysis of the changes produced. Regardless of the contrasting hormonal changes, it would seem likely that stilbestrol administration to the intact male is not unlike castration, for stilbestrol diminishes androgen production by the testicles and adrenal glands through the mechanism of reducing luteinizing hormone output by the pituitary. Experimental studies suggest that antagonistic actions may exist between estrogens and androgens in regard to the prostate.

Apparently the two methods of treatment complement each other. Nesbit and Baum in a clinical and statistical study of 1818 cases of prostatic carcinoma demonstrated that control of carcinoma of the prostate is most effectively obtained by the combined employment of castration and stilbestrol administration.

The cytological changes in the prostatic cancer cells following diethylstilbestrol therapy have been intensively studied by Kahle, Schenken and Burns. These observers were able to demonstrate certain regressive changes in the nuclear and cytoplasmic material.
Initially these changes consist of pyknosis of the nuclei and vacuolization of the cytoplasm. Later there occurs progressive fragmentation and disappearance of nuclear material, rupture of cell membranes, loss of cellular outline and finally replacement by fibrous stroma, smooth muscle and round cells.

Ludford and Dmochowski, by investigating the action of stilbestrol on ten different transplantable mouse tumors in mice of four inbred strains, were convinced that stilbestrol itself is not a mitotic poison such as colchicine and sodium cacodylate. Neither in vivo nor in vitro did stilbestrol prove to have a mitotic poisoning action on the malignant cells.

The fact that recrudescence usually occurs after varying lengths of time has been adduced by most observers as evidence that all cancer cells are not destroyed but that some remain dormant until becoming reactivated. In all cases in which radical perineal prostatectomy has been done for cancer after preoperative administration of estrogen, malignant cells have been readily demonstrated in the surgical specimens.

The Effects of Androgens.—Huggins and associates, Brendler and associates and Flocks have each reported treating three cases of carcinoma of the prostate with
testosterone propionate intramuscularly for short periods of time. Huggins states that his three cases all showed an increase in pain at the site of bony metastases. He elaborated no further. Two of Flocks' patients had bony metastases and both showed clinical deterioration on testosterone. The third case showed no effect. Two of Brendler's cases had metastases and were in relapse; they showed clinical signs of improvement as manifested by an increase in appetite and weight. Brendler's third case was unaffected. In both Flocks' and Brendler's cases which had metastases there was an increase in the level of serum acid phosphatases.

On the basis of these preliminary studies there appears to be evidence of a pluripotential response of carcinoma of the prostate to androgens. The response of patients that have had no anti-androgenic treatment is most often a worsening of their disease. Flocks' and Brendler's cases with metastases differ in that Brendler's cases were in relapse. It can be postulated that the patients treated by Brendler had neoplasms that were androgen independent and the clinical benefit noted was due to the anabolic effects of testosterone. Further investigation of the role of androgens in prostatic cancer is warranted.
Histological studies to demonstrate the effect of androgens on carcinoma of the prostate are not in the literature. However, Moore and McLellan\textsuperscript{44} gave an individual with the disease 600 mg. of testosterone propionate over a period of 12 days. The histological appearance of this cancer was not different from the usual carcinoma of the prostate.

**The Effects of Other Steroids.**—According to the concept of Reifenstein et al.,\textsuperscript{52} androgen production by the adrenals as well as by the testes is under the influence of the luteinizing hormone (LH). These workers demonstrated a decrease in the output of 17-ketosteroids following administration of methyl testosterone and adduced considerable evidence to support their point of view that the decrease depended upon reduction of LH output. Methyl testosterone is not excreted as a 17-ketosteroid due to the stability of the methyl radical in the 17-carbon position.\textsuperscript{36}

On first inspection, there appears to be little possibility that Reifenstein's concept might be utilized in the treatment of prostatic carcinoma, for methyl testosterone is a potential stimulator of the growth of prostatic carcinoma. However, Trunnel and Duffy,\textsuperscript{59}
following a similar line of reasoning, felt that progestosterone, which also decreases LH output in the female, might in the same way decrease androgen production in prostatic cancer-bearing males without itself stimulating the disease. They treated 15 patients with progesterone in doses of 25 to 300 mg. daily. Thirteen of these 15 patients responded favorably to progesterone administration; seven of these previously had a spontaneous relapse after castration or estrogen therapy. Although progesterone appears efficacious, it cannot be recommended in preference to castration or to estrogen administration. However, for patients in whom the disease has relapsed after other forms of treatment, a trial of progesterone would appear to be indicated.

Cortisone has been used in some clinics in an attempt to accomplish a "medical adrenalectomy" through the mechanism of "compensatory atrophy". Harrison, Thorn and Jenkins treated nine patients with cortisone in a dosage of 25 to 37 mg. a day for periods of one to three months after initial doses of 100 mg. for several days. All but one patient had an improved sense of well-being and some relief of pain, but there was no objective evidence of regression of neoplastic tissue. In all patients 17-ketosteroid excretion diminished on
this dose of oral cortisone, indicating a degree of adreno-
cortical inhibition. The usual fall was from approximately
10 mg. to 6 mg. in twenty-four hours on this regimen.

The general consensus is that, although cortisone
acetate reduces the 17-ketosteroid production and produces
a sense of euphoria, it does not significantly alter the
course of prostatic cancer. Cortisone administration to
the dog is not followed by androgenic effects.29

The Effects of Bilateral Adrenalectomy.--Assuming
that androgens are an activating factor in the regrowth
of prostatic cancer after castration or estrogen therapy,
Huggins and Scott32 attempted the further elimination of
androgens by total adrenalectomy. Some clinical improve-
ment was observed after this procedure in the one patient
who survived a significant length of time, 116 days, but
this was limited because of the inadequacy of the sub-
stitution therapy available at that time (1945). Cox10
made a similar effort in 1947.

The advent of adequate substitution therapy with
cortisone has reopened this line of investigation in the
past two years. Huggins and Bergenstal29 reported in
1952 the effects of bilateral adrenalectomy in seven
cases of prostatic cancer. There was one postoperative
death, and one patient died of a pulmonary embolus 49 days after adrenalectomy. The period of observation of the remaining cases at the time of their report had been four to nine months. Objective signs of improvement were: decrease in the size of the primary tumor in two of the four patients that had large, hard glands, gain in weight in all patients, increase hemoglobin concentration in five patients, increase in the total number of circulating erythrocytes in three patients, increase in the serum total proteins in three patients and the two patients who had increased levels of serum acid phosphatase showed a decrease. Pain was completely relieved in five cases two days after adrenalectomy. They were of the opinion that three of the six patients available for study in this series had had a clinical remission of the disease of a considerable magnitude.

West and associates observed the results of the procedure in seven cases with prostatic cancer. At the time of their report six of the seven patients were dead. Four of six died from their neoplastic disease; two from adrenal insufficiency. The postoperative survival period averaged 163 days. All seven patients had temporary subjective improvement averaging 83 days. Only two out of
the seven had objective signs of improvement. This lasted 90 and 133 days in the two cases.

Harrison and associates reported on seven cases of reactivated carcinoma of the prostate. In addition to the objective signs of improvement noted by Huggins in the cases that he treated by bilateral adrenalectomy, Harrison also noted relief of urinary obstruction, roentgenographic evidence of healing of osseous metastases and a decrease in the urinary 17-ketosteroid excretion in six cases. A sustained regression for 17 months has been obtained in one case.

The apparent subjective and objective improvement of patients after total adrenalectomy indicates at least a temporary biologic arrest of the progress of cancer of the prostate. Available evidence today seems to signify that this arrest is accomplished by means of altered metabolic processes pursuant to changing of the hormonal environment of the neoplastic cell.

Androgen Independence.--As defined by Huggins and Scott, the androgen independent prostatic carcinomas are those in which anti-androgenic therapy fails. Deming demonstrated the phenomenon of androgen independence by making serial transplants of prostatic cancer tissue into the anterior chamber of the eyes of guinea pigs. Up
until the eighth generation the tissue would grow only in males. After the eighth generation the tumor had so changed its biological properties that it would grow in the eyes of females or orchiectomized males. It would appear from this study that the prostatic cancer tissue employed originally required a stimulus from functioning testis for its growth in the anterior chamber of the guinea pig's eye, but with successive transfers it became sufficiently autonomous to grow without tropic stimulation.

Clinically this change in character of prostatic cancer tissue has been demonstrated by adrenalectomy in previously orchiectomized patients after their prostatic cancer had reactivated. Although the 17-ketosteroid output in the urine is greatly reduced a second regression of the tumor has not been noted in some of the cases reported that survived for a reasonable length of time suggesting that an increased production of adrenal androgen was not the cause of the regrowth of the tumor. On this evidence it has been postulated that certain prostatic cancers become independent of androgen stimulation.
FOLLOWING THE CLINICAL COURSE OF PATIENTS WITH CARCINOMA OF THE PROSTATE

A. The Serum Acid Phosphatase Test.—Prior to 1935 the physicians only laboratory aids in following the course of prostatic cancer were the microscope and X-ray. It was then discovered that the prostate was rich in an acid phosphatase, most active at a pH of five. Very little is normally present in the blood, and no excess is found in early carcinoma of the prostate which is still localized. When the tumor has extended beyond the capsule, then the amount of serum acid phosphatase shows a definite and varying increase in approximately 65 percent of cases. Excessive phosphatase is readily demonstrable in histological sections of the tumor and its metastases. However, the amount of enzyme in prostatic carcinoma tissue is less than the amount present in a normal gland and the amount found in metastatic lesions is less than in the primary tumor.

The test is of particular value in corroborating clinical and roentgenologic findings. It is of prognostic significance, in determining the response to treatment and determining when relapse has occurred. Castration or estrogen therapy will cause a decrease in the serum acid phosphatase, while injection of androgens frequently
results in a further increase; cortisone administration or bilateral adrenalectomy results in a decrease.

The test may fail in anaplastic tumors which elaborate very little of the enzyme or when blood stream or lymph invasion has not progressed sufficiently.19

B. Other Objective Evidence.—In addition to the determination of the serum acid phosphatase level there are other objective evidences that are useful in following the clinical course of prostatic carcinoma patients. In a patient who has obtained a good response to anti-androgenic therapy there is noted an improved nutritional state. This is manifested by an increase in weight, strength and activity. The blood picture shows an increase in hemoglobin, red cells and total protein.

Soft tissue masses decrease in size and osseous metastases show X-ray evidence of healing. With this decrease in size of metastases, physical findings such as neurological signs due to the metastases improve.

Increased serum alkaline phosphatase occurs in obstructive liver disease and with any significant increase in osteoblastic activity, such as occurs in Paget's disease, healing fractures, or bone reaction to metastatic
cancer. Carcinoma of the prostate with metastases to the bone usually shows both an increase in the serum alkaline and acid phosphatase. A remission of prostatic carcinoma with bony metastases is accompanied by an increase in the level of the serum alkaline phosphatase due to osteoblastic activity with healing of the osseous metastases. In carcinoma of the prostate without metastases the serum alkaline phosphatase has no prognostic significance.

An attempt has been made to correlate the clinical status of prostatic carcinoma patients with their urinary excretion of 17-ketosteroids. In the patients whose disease is controlled by estrogens, or estrogens and orchietomy, there is a significant, persistent decrease in the urinary excretion of 17-ketosteroids. Orchietomy alone results in a temporary decrease but the value soon returns to normal or slightly higher.\textsuperscript{12} The attempt has been made to ascertain a rise in 17-ketosteroid excretion as a signal of increased adrenal activity and consequent reactivation of cancer because of escape from hormonal control.\textsuperscript{24} Such a correlation of increased 17-ketosteroid excretion and coincident reactivation of prostatic cancer would be an important observation but so far it has not been demonstrated.

Recent experimental investigations of the glycolytic
enzyme, aldolase, has demonstrated that any change in prostatic cancer activity is reflected by a prompt fluctuation in the level of serum aldolase. Abnormally high concentrations of aldolase are present in the serum of 60 to 70 percent of patients studied with active, advanced, untreated prostatic carcinoma. Following orchiectomy or stilbestrol administration the increased serum aldolase concentration returns to normal range in 85 to 90 percent of the patients studied. Baker$^4$ and associates, therefore, believe that serum aldolase is equally as effective in indicating activity as is the classically accepted biochemical indicator of advanced prostatic cancer activity, namely, serum acid phosphatase. The value of this test in cancer of the prostate is yet to be proven; only preliminary investigations have been made.

Malcolm and Rusche$^{42}$ working with proteolytic enzymes and their inhibitors demonstrated that chymotrypsin and rennin inhibitors of the serum provide a sensitive method for gauging the therapeutic control of neoplasms. In a study of six patients with carcinoma of the prostate they found a correlation to exist between the levels of these two enzymes in the serum and the clinical status of the patients. In patients that showed a good response to anti-androgenic therapy, chymotrypsin inhibitor tended to
rise and rennin inhibitor fell. In patients showing a reactivation of their disease, the serum level of anti-
rennin rose and anti-chymotrypsin fell. Adenocarcinoma of the prostate which is limited to the confines of the capsule has not as yet been found to induce an imbalance in the enzyme pattern.

Their tentative assumption is that, in some manner, chymotrypsin inhibitor reflects the rate of protein degradation while rennin inhibitor seems to be allied with protein synthesis. They believe that the degree of control obtained in prostatic carcinoma following orchiectomy or estrogen therapy may be measured objectively by observing the resulting changes in the concentration of the two factors.

OWN INVESTIGATIONS

On the basis of experimental and clinical investigations many urologists believe that relapse in carcinoma of the prostate that has been controlled by hormonal measures is due to a restimulation of residual cancer by some androgenically active substances produced by the adrenal glands. Since the number of circulating eosinophils fall sharply when the adrenal gland is acutely stimulated, it seemed possible that such an increase in
adrenal function might be reflected in the eosinophil count. The purpose of this investigation was to determine whether or not such a change occurred in the circulating eosinophils of patients with reactivated carcinoma of the prostate. Quantitative eosinophil leucocyte determinations were done in the following groups of patients:

1. Patients with carcinoma of the prostate who were controlled by hormonal therapy.

2. Patients with reactivated carcinoma of the prostate after a remission induced by anti-androgenic measures.

3. Patients with a type of malignancy other than carcinoma of the prostate.

Methods.—The technique employed in counting the eosinophil leucocytes was described by T. G. Randolph in 1943. Freely flowing capillary blood was collected from a deep puncture in the tip of the finger. The blood was mixed in a proportion of 1:20 with a stain composed of diluting fluid containing 0.1 percent methylene blue and 0.1 percent of phloxine dissolved in an equal part of propylene glycol and water. The eosinophil granules assume a deep red color and the nucleus a light robin egg blue. The remainder of the white blood cells stain a light greenish hue. The red blood corpuscles are
hemolyzed. A sample of the bloodstain mixture was immediately transferred to a counting chamber where the main staining occurs in the course of fifteen minutes. The cells in an entire Spencer counting chamber were enumerated and the number obtained multiplied by the factor 11.1 to obtain the number of eosinophils per cu. mm. of blood.

Many standard textbooks of clinical pathology and hematology present the normal range of eosinophils in the circulating blood in health. The range of normal is estimated to be 0-400 by Osgood, 50-400 by Todd and Sanford, and 75-300 by Kracke and Parker. At the time this study was undertaken, 1950, the literature did not reveal any significant data regarding the fluctuations or diurnal variations of such blood cells in health. Since that time, however, Bonner demonstrated that fasting alone will cause a drop in the eosinophil count. Halberg and associates found an average morning drop in eosinophils between the hours of 06:30 and 09:30 of from 430 to 251 per cu. mm. controlled subjects with the count returning toward the higher level during midday. They found a rough inverse relation between oral temperature and the eosinophil level in controlled subjects. Their observations failed to show the normal diurnal rhythm in patients with Addison's disease, bilateral adrenalectomy
and hypopituitarism. From their studies they have concluded that the adrenal control hormones play a part in eosinophil level fluctuations in the subject. Fisher and Fisher in 463 observations on 170 subjects found a diurnal variation in both fasting and non-fasting individuals. Of the fasting group, 23 percent showed a drop of 40 percent or more in the total circulating eosinophils in the forenoon with a rise later in the afternoon. The non-fasting subjects demonstrated this phenomenon fairly consistently but not to the degree observed in the fasting subjects. They concluded that "it would be difficult to use this phenomenon as a sensitive indicator of pathologic variations".

The counts for this study were carried out on patients being followed in the Urology Division of the Bonfils Tumor Clinic of the University of Colorado Medical Center. Counts taken on patients with types of malignancy other than cancer of the prostate were made while the patients were hospitalized at Colorado General Hospital or while they were being followed in the Surgery Division of the Bonfils Tumor clinic. The eosinophil counts were made approximately every two months. Due to the fact that some patients have to come great distances and make infrequent visits to the clinic only one or two counts were made in
some instances. The counts were performed on ambulatory patients under basal conditions, without food since breakfast and resting in the laboratory. The taking of samples commenced at approximately 12:00 p.m. This time of day in relation to the diurnal fluctuations of the circulating eosinophils finds them near their high level of normal.

Table I gives the results of eosinophil leucocyte counts, one enumeration, in ten normal young adults. Healthy subjects in an age group similar to the usual age of patients with carcinoma of the prostate could not be obtained.

Table II demonstrates counts in two patients with three-year cures of cancer of the prostate following radical prostatectomy. There is no evidence of disease in either patient, therefore I think these counts could be considered normals in a healthy older age group.

When surgery for complete removal of the tumor is precluded due to local extension or metastases, treatment in this clinic consists of transurethral electroresection of the prostate for relief of urinary obstruction and anti-androgenic therapy—orchiectomy and stilbestrol 5 mg. orally, daily. This method of treatment has been followed routinely in this clinic since Nesbit's and Baum's
### TABLE I

**Eosinophil Leucocyte Counts in Ten Healthy Young Adults**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Count</th>
</tr>
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<tbody>
<tr>
<td>P.J.</td>
<td>20</td>
<td>143</td>
</tr>
<tr>
<td>D.A.</td>
<td>29</td>
<td>275</td>
</tr>
<tr>
<td>V.D.</td>
<td>27</td>
<td>199</td>
</tr>
<tr>
<td>I.M.</td>
<td>33</td>
<td>353</td>
</tr>
<tr>
<td>I.A.</td>
<td>21</td>
<td>198</td>
</tr>
<tr>
<td>V.P.</td>
<td>24</td>
<td>246</td>
</tr>
<tr>
<td>G.P.</td>
<td>25</td>
<td>253</td>
</tr>
<tr>
<td>D.S.</td>
<td>23</td>
<td>289</td>
</tr>
<tr>
<td>A.W.</td>
<td>32</td>
<td>165</td>
</tr>
<tr>
<td>D.H.</td>
<td>28</td>
<td>246</td>
</tr>
</tbody>
</table>

The average of this group is 237 eosinophils per cu. mm. of blood. A variation of from 165 to 353 eosinophil leucocytes per cu. mm. is observed. On the basis of Fisher and Fisher's work they believe, for clinical purposes, that a normal range of 25 to 300 would be optimal.
TABLE II

Eosinophil Leucocyte Counts in Two Patients Apparently Cured by Radical Perineal Prostatectomy

<table>
<thead>
<tr>
<th>Pt.</th>
<th>Age</th>
<th>Counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.H.</td>
<td>71</td>
<td>154 110 220 220</td>
</tr>
<tr>
<td>C.S.</td>
<td>72</td>
<td>166 143 144 121</td>
</tr>
</tbody>
</table>

The counts in these two patients closely resemble the counts in the healthy young adults.

Insufficient numbers of patients were available for study to warrant a vigorous statistical evaluation of the results. It is obvious, however, that if a depression in eosinophil count is to be of clinical value in following
report in 1950 indicated that carcinoma of the prostate is most effectively controlled by the combined employment of castration and stilbestrol administration. When necessary a punch biopsy is done to establish the diagnosis. All of the cases of carcinoma of the prostate to be presented subsequently had had a histological diagnosis of their disease and clinical management has been carried out as indicated above.

Eight cases of carcinoma of the prostate were observed during reactivation of their disease. Six of these cases had metastases. All of these patients showed some evidence of clinical deterioration. (See Table III)

With the exception of three observations in different patients, all of the eosinophil leucocyte counts in the patients with reactivated prostatic carcinoma are below 100 per cu. mm. of blood. The normal range of eosinophils in the circulating blood is so great that the arbitrary value of 100 has been chosen for purposes of comparison between the different groups of patients in this study.

Insufficient numbers of patients were available for study to warrant a vigorous statistical evaluation of the results. It is obvious, however, that if a depression in eosinophil count is to be of clinical value in following
TABLE III

Eosinophil Counts in 8 Patients with Cancer of the Prostate who were Relapsing after Previously being Controlled by Orchiectomy and Stilbestrol.

<table>
<thead>
<tr>
<th>Pt.</th>
<th>Age</th>
<th>Eosinophil Counts</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.W.</td>
<td>67</td>
<td>99 110 99</td>
<td>Fullblown regression during entire period of time covered by counts.</td>
</tr>
<tr>
<td>D.W.</td>
<td>79</td>
<td>66 77</td>
<td>Died five months after diagnosis, two months after last count.</td>
</tr>
<tr>
<td>C.P.</td>
<td>86</td>
<td>55 44 88 77 99</td>
<td>Relapse only apparent at time of last visit to the clinic.</td>
</tr>
<tr>
<td>J.E.</td>
<td>66</td>
<td>55 77 99</td>
<td>Symptoms of relapse at time of last count.</td>
</tr>
<tr>
<td>A.P.</td>
<td>82</td>
<td>55</td>
<td>Died three months after count with metastases to penis.</td>
</tr>
<tr>
<td>J.C.</td>
<td>81</td>
<td>88 0 22</td>
<td>Pathological fracture of the left hip.</td>
</tr>
<tr>
<td>J.S.</td>
<td>75</td>
<td>165 88</td>
<td>Confined to bed. No known metastases.</td>
</tr>
<tr>
<td>L.E.</td>
<td>57</td>
<td>198</td>
<td>Very anaplastic tumor showing rapid growth locally. No known metastases.</td>
</tr>
</tbody>
</table>

It is noted that the counts in this group have a tendency to be consistently lower than those seen in healthy individuals. The average is 80.5 eosinophil leucocytes per cu. mm. of blood.
the course of prostatic carcinoma it must occur with regu-
larly and be of considerable magnitude in patients who
have reactivated disease. To determine if there was a dif-
terence in the eosinophil leucocyte count between patients
with carcinoma of the prostate showing reactivation of their
disease and patients that were controlled by anti-androgenic
therapy, serial quantitative eosinophil leucocyte determina-
tions were done on a group of 27 patients exhibiting a good
response to stilbestrol and orchiectomy. Only three of this
group of 27 patients had metastases. (See Table IV)

The three cases with metastases were well controlled
by anti-androgenic therapy. With the exception of one
determination all of the counts in these three patients
were above 100 eosinophil leucocytes per cu. mm. of blood.
The last eight subjects listed in this group had counts
that were all below 100. The determinations in this group
of eight patients demonstrate the presence of a great
degree of overlap in the eosinophil counts between the
reactivated and the controlled group of cases. The
patients with low counts showed no evidence of a reactiva-
tion of their disease.

Because of the marked degree of overlap noted be-
tween the reactivated and controlled cases this labora-
tory procedure does not seem to be very promising as a
TABLE IV

Eosinophil Enumerations in 27 Patients with Carcinoma of the Prostate Who were Well-controlled on Therapy Consisting of Orchiectomy and Stilbestrol, 5 Mg. Daily.

<table>
<thead>
<tr>
<th>Pt.</th>
<th>Age</th>
<th>Eosinophil Counts 8 week Intervals</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.B.</td>
<td>73</td>
<td>203 198 144 198 209</td>
<td></td>
</tr>
<tr>
<td>J.G.</td>
<td>67</td>
<td>355 308 275 209 132 275</td>
<td></td>
</tr>
<tr>
<td>F.H.</td>
<td>75</td>
<td>264 220 154 133 198</td>
<td></td>
</tr>
<tr>
<td>J.R.</td>
<td>67</td>
<td>133 220 164 110 200</td>
<td></td>
</tr>
<tr>
<td>M.S.</td>
<td>66</td>
<td>297 198 143</td>
<td></td>
</tr>
<tr>
<td>B.V.</td>
<td>70</td>
<td>191 132 110 110</td>
<td></td>
</tr>
<tr>
<td>M.M.</td>
<td>77</td>
<td>154 99 110</td>
<td></td>
</tr>
<tr>
<td>H.M.</td>
<td>75</td>
<td>210 143 110 154 210</td>
<td></td>
</tr>
<tr>
<td>W.N.</td>
<td>59</td>
<td>170 132 110 148</td>
<td></td>
</tr>
<tr>
<td>H.D.</td>
<td>75</td>
<td>66 66 121</td>
<td></td>
</tr>
<tr>
<td>A.K.</td>
<td>69</td>
<td>99 88 99 110 154</td>
<td></td>
</tr>
<tr>
<td>J.R.</td>
<td>61</td>
<td>33 44 33 144</td>
<td></td>
</tr>
<tr>
<td>R.M.</td>
<td>67</td>
<td>154 44 99</td>
<td></td>
</tr>
<tr>
<td>J.H.</td>
<td>73</td>
<td>154</td>
<td></td>
</tr>
<tr>
<td>S.P.</td>
<td>74</td>
<td>132 110 154</td>
<td>Leukotomy for pain due to metastases 3 yrs. before counts.</td>
</tr>
<tr>
<td>F.B.</td>
<td>70</td>
<td>198 121</td>
<td></td>
</tr>
<tr>
<td>H.K.</td>
<td>67</td>
<td>112 99</td>
<td></td>
</tr>
<tr>
<td>M.F.</td>
<td>74</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>A.G.</td>
<td>71</td>
<td>142</td>
<td></td>
</tr>
<tr>
<td>J.D.</td>
<td>84</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>A.E.</td>
<td>84</td>
<td>55 44 44 55 99</td>
<td></td>
</tr>
<tr>
<td>E.S.</td>
<td>82</td>
<td>66 35 77 77 55</td>
<td></td>
</tr>
<tr>
<td>A.T.</td>
<td>81</td>
<td>99 66 88 77</td>
<td></td>
</tr>
<tr>
<td>F.O.</td>
<td>91</td>
<td>88 44 44 0 99</td>
<td></td>
</tr>
<tr>
<td>B.M.</td>
<td>71</td>
<td>44 55</td>
<td></td>
</tr>
<tr>
<td>T.S.</td>
<td>74</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>J.M.</td>
<td>79</td>
<td>99</td>
<td></td>
</tr>
</tbody>
</table>

The average eosinophil leucocyte count in this group is 124.8 per cu. mm. of blood. The last 8 subjects listed in the table had counts that were consistently below 100. In addition, six others had a count of less than 100 at one or more times.
tool for following reactivation of cancer of the prostate. To see if the suggestively low counts in the reactivated group were unique for cancer of the prostate, patients with other metastatic carcinoma of other organs were investigated. Table V illustrates a group of patients with various types of metastatic malignancies other than carcinoma of the prostate. Many of these patients were nearing terminus. Although the number of determinations are inadequate it would appear that the eosinophil counts are lower than the counts of the controls or the patients with prostatic carcinoma controlled by hormone therapy.

When the results of the eosinophil leucocyte determinations in the patients with metastatic cancer other than cancer of the prostate are compared to the results obtained in the patients with reactivated prostatic carcinoma, it is observed that the two groups have eosinophil counts that are very similar. This observation suggests that the low counts in both groups are due to adrenal hyperactivity caused by "non-specific" stress and indicates that a low eosinophil count is not specific in reactivated cancer of the prostate. The comparison of the results in the three groups of patients studied indicates that the absolute level of circulating eosinophils is not a good gauge of prostatic cancer activity, and that

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Counts</th>
<th>Counts</th>
<th>Counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer of kidney--before surgery</td>
<td>68</td>
<td>80</td>
<td>77</td>
</tr>
<tr>
<td>Cancer of breast</td>
<td>69</td>
<td>80</td>
<td>77</td>
</tr>
<tr>
<td>Cancer of brain</td>
<td>68</td>
<td>79</td>
<td>77</td>
</tr>
<tr>
<td>Cancer of uterus</td>
<td>67</td>
<td>78</td>
<td>77</td>
</tr>
<tr>
<td>Cancer of lung</td>
<td>68</td>
<td>79</td>
<td>77</td>
</tr>
</tbody>
</table>

**Table V**

Eosinophil Counts in 20 Patients with Metastatic Malignancies Other Than Cancer of the Prostate.
### TABLE V
Eosinophil Counts in 20 Patients with Metastatic Malignancies Other Than Cancer of the Prostate

<table>
<thead>
<tr>
<th>Pt.</th>
<th>Age</th>
<th>Eosinophil Counts</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.H.</td>
<td>71</td>
<td>71 60 44</td>
<td>Cancer of bladder pre- and postoperative.</td>
</tr>
<tr>
<td>F.E.</td>
<td>70</td>
<td>99 110</td>
<td>Cancer of stomach—inoperable.</td>
</tr>
<tr>
<td>A.S.</td>
<td>73</td>
<td>77</td>
<td>Cancer of kidney—before surgery.</td>
</tr>
<tr>
<td>F.D.</td>
<td>59</td>
<td>55</td>
<td>Cancer of lung—inoperable.</td>
</tr>
<tr>
<td>T.W.</td>
<td>68</td>
<td>22</td>
<td>Cancer of stomach—inoperable.</td>
</tr>
<tr>
<td>L.P.</td>
<td>65</td>
<td>177 143</td>
<td>Cancer of cervix—inoperable.</td>
</tr>
<tr>
<td>J.P.</td>
<td>81</td>
<td>151</td>
<td>Cancer of cervix—recurrent.</td>
</tr>
<tr>
<td>K.L.</td>
<td>51</td>
<td>888 165</td>
<td>Cancer of cervix—recurrent.</td>
</tr>
<tr>
<td>C.P.</td>
<td>71</td>
<td>11</td>
<td>Cancer of cervix—inoperable.</td>
</tr>
<tr>
<td>R.W.</td>
<td>52</td>
<td>99</td>
<td>Cancer of cervix—recurrent.</td>
</tr>
<tr>
<td>J.C.</td>
<td>62</td>
<td>0</td>
<td>Expired two days after count.</td>
</tr>
<tr>
<td>M.M.</td>
<td>42</td>
<td>88</td>
<td>Cancer of cervix—inoperable.</td>
</tr>
<tr>
<td>T.M.</td>
<td>60</td>
<td>99 66</td>
<td>Metastatic cancer of breast.</td>
</tr>
<tr>
<td>R.M.</td>
<td>76</td>
<td>88</td>
<td>Metastatic cancer of breast.</td>
</tr>
<tr>
<td>E.H.</td>
<td>65</td>
<td>66</td>
<td>Metastatic cancer of breast.</td>
</tr>
<tr>
<td>A.F.</td>
<td>48</td>
<td>187 144</td>
<td>Metastatic cancer of breast.</td>
</tr>
<tr>
<td>E.M.</td>
<td>45</td>
<td>110</td>
<td>Cancer of breast—preoperative.</td>
</tr>
<tr>
<td>B.P.</td>
<td>72</td>
<td>132 110 99</td>
<td>Cancer of breast—one year cure.</td>
</tr>
<tr>
<td>F.S.</td>
<td>61</td>
<td>55</td>
<td>Metastatic cancer of breast.</td>
</tr>
</tbody>
</table>

Counts in this group of patients have a tendency to be low when compared to the counts in the patients with cancer of the prostate who were controlled by orchiectomy and stilbestrol. The average count is 88.1. Sixteen had at least one count of less than 100 eosinophil leucocytes per cu. mm. of blood.
quantitative eosinophil determinations are of no value in following the clinical course of patients with carcinoma of the prostate.

**SUMMARY AND CONCLUSIONS**

The history of the development of hormone therapy of carcinoma of the prostate has been reviewed. Endocrine factors associated with cancer of the prostate and methods of following the clinical course of patients with the disease have been presented.

A quantitative determination of the circulating eosinophil leucocytes has been done in a group of 55 patients with carcinoma. Of these patients 35 had carcinoma of the prostate. Twenty-seven of these 35 patients were controlled by anti-androgenic therapy and the remaining eight cases of cancer of the prostate were in relapse after previously being controlled by anti-androgenic measures. The other 20 cancer patients in which counts were done had a metastatic malignancy of a type other than cancer of the prostate. An attempt was made to correlate the clinical status of each patient with that patient's eosinophil counts.

The arbitrary value of 100 eosinophil leucocytes per cu. mm. of blood has been chosen for a basis of com-
parison among the controlled cases, the reactivated cases and the cases with other metastatic malignancies. The average eosinophil count in the group of 27 prostatic carcinoma patients controlled by anti-androgenic therapy was 124.8. In this group there were eight patients who had eosinophil determinations that were in every instance below 100 per cu. mm. of blood. In the eight patients showing reactivation of their prostatic cancer, 20 eosinophil determinations were done. The results were above 100 in only three determinations. The average in this group was 80.5 eosinophil leucocyte per cu. mm. of blood. The eosinophil determinations in the group of 20 patients with a metastatic malignancy other than prostatic cancer averaged 88.1. There were 16 of these patients that had one or more counts below 100.

The great degree of overlap noted in the counts in the three groups of cases, a small magnitude of the reduction of the eosinophil counts in the reactivated cases (approximately 30 percent) and the similarity of the counts in the group with reactivated prostatic cancer and the group with other types of metastatic malignancies indicate that the eosinophil leucocyte count is probably not a reliable method of following the clinical course of carcinoma of the prostate.
When this study was undertaken in 1950 the exact relationship of the circulating eosinophils to the adrenal gland was not fully appreciated. Because the administration of gluco-corticoids or of ACTH to individuals with functional adrenals resulted in a decrease in the number of circulating eosinophils, it was hoped by many that the number of circulating eosinophils might be an accurate reflection of adrenal function at all times. Since that time most investigators have come to feel that such is not the case, except possibly that very low eosinophil counts are very frequently encountered in patients during periods of known adrenal hyperfunction such as occurs in the post-operative period and at other times of severe stress. From this study it would appear that cancer patients undergoing relapse after previously being controlled by hormonal or surgical therapy exhibit a low eosinophil count. This observation might suggest that a depressed eosinophil count is an indication of adrenal response to "non-specific" stress rather than adrenal hyperfunction with increased androgen stimulation to the tumor. This supposition is strengthened by the observation that the eosinophil count is consistently low in the group of patients nearing terminus with metastatic malignancies other than carcinoma of the prostate.
From this review and study it appears to me that if continuing progress is to be made in the investigation and treatment of prostatic cancer, the exact role of androgens in the induction of cancer or the maintenance of cancer growth must be ascertained. Emphasis must be placed on the necessity of classifying prostatic cancers according to their biological properties before drawing conclusions as to the effectiveness of different modes of therapy. If androgen dependence is all-important, other means of neutralizing or eliminating androgens must be explored. If other factors exist, these in turn must be investigated.


REFERENCES


