BILATERAL ADRENALECTOMY IN PROSTATIC CANCER

CLINICAL FEATURES AND URINARY EXCRETION OF 17-KETOSTEROIDS AND ESTROGEN*

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METHODS which inhibit androgenic production or action either by orchiectomy or estrogen administration have been found to reduce the activity of cancer of the prostate in many instances[1, 2]; in other cases these measures fail to control the disease, either immediately or more often following a remission of some months or years. It has been postulated that the production of androgen by organs other than the testes is responsible for the activity of prostatic cancer where control of the testicular androgens has resulted in failure, either immediate or delayed.

There is considerable evidence that the adrenal cortex of several species, including man, elaborates androgens in amounts of physiologic significance. Androgen-producing tumors occurring in the adrenal cortex, especially of women and little boys, often lead to masculinization—regression of the pathologic status occurring after extirpation of the tumor. The X-zone of Price[3] occurring in the adrenal cortex of young rats induces pubertal changes in the prostate of males castrated early in life. In guinea-pigs which had been castrated shortly after birth, tumors of the adrenal cortex developed after many months, inducing maturity in the secondary sex organs[4]; Wooley and Little[5] observed that early orchiectomy in male mice of the strain JAX ce produced adrenal tumors with masculinization in some and estrogenic effects in the accessory sex organs of others. After orchiectomy for prostatic cancer there is a fall in the urinary excretion of total neutral, as well as *beta,* 17-ketosteroids followed by a considerable increase for prolonged periods.[19] This preliminary fall appears to be dependent upon the removal of the gonads and is not an indifferent effect of the operative procedure. Further, it seems likely that the increase is induced through the adrenal cortex by way of the pituitary gland.

The study of prostatic cancer has been hampered by the rarity of this disease in laboratory animals. Adrenocarcinoma of the prostate gland has not been produced in the laboratory; the implantation of 1:2 benzpyrene in the prostate of rats[6] has resulted in the production of squamous cell carcinoma, a type which is rare in man. Prostatic cancer occasionally occurs spontaneously in the dog. Schlotthauer and Millar[7] observed it in three dogs, and Krause,[8] and Roth,[9] in single cases. Engle and Stout[10] discovered prostatic cancer in a

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monkey at autopsy. We have observed adenocarcinoma of the prostate twice in a series of 637 dogs whose prostate glands were examined cytologically in this laboratory. The disease has never been recognized in a living laboratory animal so that functional observations have not been feasible in the lower forms.

In experimental animals the adrenal glands have been removed with preservation of life and good health for many months by means of substitution therapy. Because of the hypothesis that adrenal androgens were stimulating the growth of prostatic cancer and since the natural life expectancy clearly was short in the cases to be considered in this paper, the adrenal glands were removed in two stages from four men with rapidly growing metastatic cancer of the prostate, all having had the previous benefit of orchiectomy. These men survived 1.5, 1.5, 11 and 116 days after bilateral adrenalectomy, and are the basis of the present communication. In this paper it will be demonstrated that there is an additional factor in some prostatic cancers, namely, androgen-independence.

Since postoperative adrenal failure occasionally occurs following less drastic adrenal surgery, the methods by which survival occurs after complete adrenalectomy are presented in detail. Complete adrenalectomy in man, with survival, has not been described previously.

CASE REPORTS

Case 1.—A. E. D. (history No. 266,424), a physician, age 58, was first seen on July 9, 1941, complaining of sciatica of six months' duration. The prostate gland was hard, nodular, fixed and very large. A roentgenogram showed widespread osteoplastic metastases in the pelvis. The serum acid phosphatase was 7.5 units* and alkaline phosphatase 36 units.

A diagnosis of cancer of the prostate was made, and bilateral orchiectomy was accordingly carried out on July 17, 1941. Relief of pain occurred within six days, and shortly thereafter the patient resumed the practice of his profession. Seven months after orchiectomy, acid and alkaline phosphatase values were 4.5 and 6 units respectively. One month later a reactivation of the disease occurred, judging by a rise of alkaline phosphatase, which was progressive. In June, 1942, pain reappeared and became steadily worse, requiring injections of morphine. On January 25, 1943, the left adrenal gland was removed through a loin incision; its weight was 5.4 Gm., and it was normal on cytologic study. The blood pressure was 120/84. The serum sodium and potassium were normal. On February 12, 1943, the right adrenal was removed under ethylene anesthesia; it was normal in appearance and weighed 5.5 Gm. The blood pressure was unchanged for 12 hours when it decreased to 70/60. The temperature rose to 38.2° C. eight hours after operation, rising to 40° C. at 20 hours. The patient died in shock 36 hours after removal of the second adrenal. At no time were the values for serum sodium and potassium abnormal, and the blood sugar ranged from 82 to 131 mg. per cent.

Prior to castration the total neutral urinary 17-ketosteroids ranged from 5 to 7 mg. per 24 hours (Fig. 1). Following orchiectomy there was no immediate change in these values, but from day 13 to 20, the 17-ketosteroids fell to less than the preoperative level; however, over the course of a few months to a year and a half they gradually rose to

*All phosphatase results are expressed in King and Armstrong units per 100 cc. of serum.

12 mg. per day, almost twice the level found prior to castration. Following removal of the left adrenal gland there was a moderate decrease in 17-ketosteroid excretion; following right adrenalectomy there was a sharp reduction to a level of 2.0 mg. for the first 24 hours. Obviously, this patient did not live long enough for us to make any significant observations either clinically or chemically except that the treatment of acute adrenal insufficiency was inadequate.

Special therapy in this case included adrenal cortical extract* 35 cc. intramuscularly on day 0 minus 1† 50 cc. on 0 day; and 50 cc. on 0 plus 1. Desoxycorticosterone acetate (DCA)‡ was injected in amounts of 5 mg. on 0 day and 20 mg. on 0 plus 1. A transfusion of plasma, 600 cc. was given on 0 plus 1.

Autopsy revealed adenocarcinomas of the prostate with metastases to lymph nodes and bone.

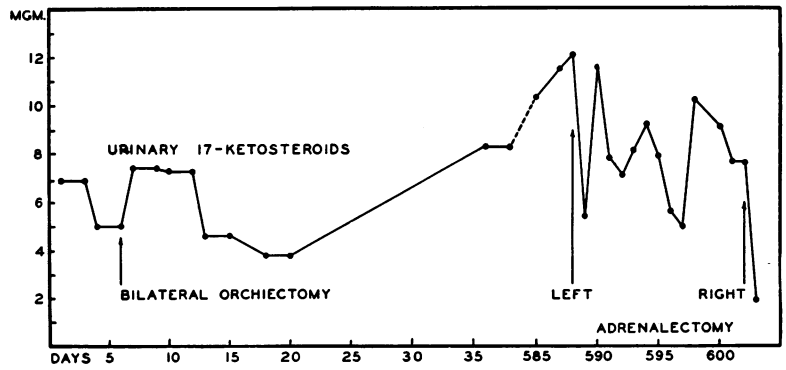

FIG. 1.—Excretion of total urinary 17-ketosteroids following orchiectomy and adrenalectomy, Case 1.

Case 2.—W. E. B. (history No. 312,344), a clerk, age 60, was first seen on June 23, 1943, complaining of pain in the lower back and in both legs for one month. For several years there had been urinary frequency. The prostate gland was large (4+), hard and nodular, and acid and alkaline phosphatase values were 3.75 and 24 units. A diagnosis of carcinoma of the prostate was made and bilateral orchiectomy performed on July 1, 1943, with complete relief of pain for five months, so that the patient resumed his work. The prostate became soft and atrophic except for slight induration in the left lobe. In December, 1943, sciatic pain recurred and became severe.

The patient was readmitted on May 7, 1944. The prostate gland was much smaller than before orchiectomy; the right lobe was atrophic and soft, the left lobe contained a hard nodule of walnut size. The blood pressure was 155/85. The left adrenal, weighing 5 Gm., was removed on May 12; the right adrenal, weighing 4.5 Gm., was excised on June 2, 1944.

Following complete adrenalectomy the temperature rose to 38.7° C. within eight hours and continued to rise (Fig. 2). Systolic blood pressure remained between 130 and 160 millimeters for 36 hours when it fell to 70 millimeters. The pulse was feeble and rapid, and the urinary output was low. The patient died in shock 54 hours after

* Adrenal cortical extract used was the 10 per cent alcoholic extract of the adrenal glands, and was prepared by the Upjohn Company and the Wilson Laboratories.

† 0 minus 1 signifies the day before the operation; 0 is the day of operation, *etc.*

‡ We are indebted to Dr. Erwin Schwenk of Schering Corporation for the gift of this material.

adrenalectomy. Some degree of adrenal insufficiency followed the removal of the first adrenal gland as reflected by a lowering of serum sodium to 134 m. eq. per liter and an elevation of the serum potassium to 5.6 m. eq. per liter. These values for sodium were the lowest obtained for this patient and were determined on May 29, 1944. However, by the morning of June 2, 0 day, the values for sodium and potassium were normal, 141.2 and 4.3 m. eq. per liter, respectively.

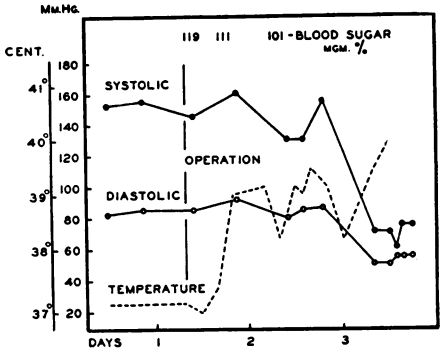

FIG. 2.—Decrease of blood pressure and increased body temperature in adrenal insufficiency of surgical type, Case 2.

Special therapy in this case included DCA, 10 mg. on days 0 minus 1, 0 day, 0 plus 1 and 0 plus 2; adrenal cortical extract 45 cc. on 0 day and 0 plus 2.

Autopsy revealed an undifferentiated carcinoma of the prostate, which appeared degenerated in many areas, with metastases to bones and lungs.

Case 3.—G. T. R. (history No. 269,817), a clerk, age 56, was first seen September 4, 1941, when he complained of urinary frequency for nine months and sciatica in the left leg for six weeks. The prostate was convex, hard and nodular, with extracapsular infiltration, but roentgenograms of the pelvis were interpreted as normal. Acid

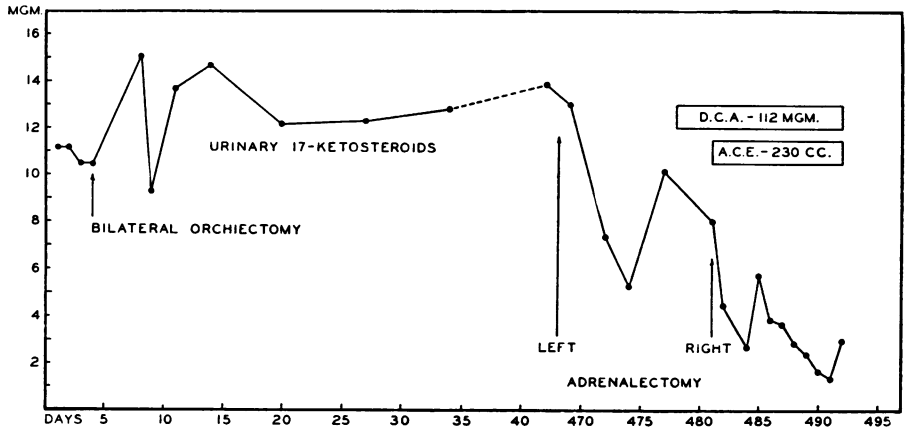

FIG. 3.—Decrease of total urinary 17-ketosteroids after adrenalectomy, Case 3.

and alkaline phosphatase values were 43 and 16 units respectively. Since the diagnosis of cancer of the prostate was certain, the testes were removed on September 16, 1941. The symptoms were improved and the patient returned to work. One year postoperatively the serum phosphatases were normal; the patient felt well and the prostate was soft, atrophic and without clinical evidence of carcinoma. One month later sciatica appeared in the right leg and rapidly became severe and required morphine and the patient was readmitted on December 22, 1942. At that time the prostate was atrophic, and the blood pressure was 134/72. Roentgenograms showed osteoplastic metastases in the pelvis. Acid and alkaline phosphatase values were 7 and 40 units. The left adrenal, weighing 5.5 Gm., was removed on December 30, 1942. The right adrenal weighed 6.5 gm. at removal on January 11, 1943. The temperature rose to 39.4° C. within four

hours thereafter and the blood pressure fell to 70/40 in 16 hours. The postoperative period was very stormy. During the early postoperative period the blood pressure and the urinary output were low; later the systolic blood pressure was increased to 150 millimeters and the daily output of urine was increased, but the patient became edematous, his fever persisted, and he died on 0 plus 11. The serum sodium and potassium were respectively 143 and 5.6 m. eq. per liter on 0 plus 8. Alkaline phosphatase values decreased from 40 to 11 units during the period of survival after complete adrenalectomy.

Preliminary total urinary 17-ketosteroids ranged from 10 to 12 mg. per 24 hours. Unlike the majority of patients we have studied these values did not undergo a preliminary decrease after orchiectomy, but rose instead, the rise being sustained for a period of 470 days (Fig. 3). After removal of the left adrenal there was a gradual fall in urinary ketosteroids; after removal of the right they continued to fall reaching their lowest value of 1.4 mg. per day on 0 plus 9 day. These values for 17-ketosteroids occurred in spite of the administration of desoxycorticosterone acetate and adrenal cortical extract. Comb-growth androgens determined before either adrenal was removed were of the order of 25 I. U. per day. Zero values were obtained on 0 plus 9 day indicating total absence of androgen excretion.

At autopsy, carcinoma of the prostate was found; there was marked regression of the primary tumor associated with very malignant metastases to bone.

Case 4.—F. A. (history No. 315,065), a blond laborer, age 45, was first seen on August 4, 1943, complaining of progressive urinary frequency and dysuria for one year. The prostate was firm, nodular and fixed. Acid and alkaline phosphatase values were 6 and 11 units, respectively, and roentgenograms showed a normal pelvis. On August 11, 1943, the testes were removed, and a biopsy of the gland through a perineal incision revealed an undifferentiated cancer of the prostate. For the next eight months the patient was relieved of his symptoms. The nodularity and induration of the prostate disappeared.

Ten months after orchiectomy the patient developed sciatica and began to lose weight, amounting to 10 kg. by the end of the 14th month. The sciatic pain became severe and morphine was prescribed. He developed edema of the thighs.

On November 11, 1944, the left adrenal gland, weighing 5 Gm., was removed, under spinal anesthesia, through a loin incision, and the right adrenal gland, weighing 5 Gm., was similarly excised on December 4, 1944. The immediate convalescence was uneventful; blood pressure was maintained at the preoperative level and there was a fever of 38° C. only on two days, 0 plus 4 and 0 plus 8. The details of pre- and postoperative treatment are given in Table I. The patient walked on the 8th day. The sciatic pain was relieved after operation but the appetite was poor. The serum sodium fell to 132 m. eq. per liter after removing the first adrenal, and at this time the serum potassium was 5.6 m. eq. per liter. There was no further reduction in serum sodium or potassium following complete adrenalectomy. Two months after the remaining adrenal was excised, the serum sodium was 140.5 and the serum potassium 5.0 m. eq. per liter.

On December 20, 1944 (0 plus 16) brown-black pigment was noted under the eyes and beneath adhesive strapping in the loin areas; the prostatic bed was soft and smooth and presented no clinical evidence of carcinoma, and the patient was discharged from the hospital. Pigmentation increased in the fingers, external genitalia and near the incisions, especially around the site of the drains, skin sutures and where adhesive tape had been located.

There followed a period of poor health featured by lack of appetite, occasional vomiting and loss of weight. On February 7, 1945, urinary retention occurred which was treated by inlying catheter. Pubic and axillary hair became sparse. A decrease of pain, estimated at 75 per cent, occurred following adrenalectomy, but opiates were still required. During the last two weeks of life there was recurrence of slight edema of the legs. The patient died 116 days after complete adrenalectomy.

The urea clearance was 45.9 cc. (85 per cent of mean normal) three weeks before death, when the patient was receiving 25 mg. of DCA daily, and phenolsulfonephthalein excretion in two hours was 85 per cent of the amount injected; blood urea was 9.3 mg. per cent. The red blood count was 4.3 millions on January 14, 1945, 3.52 millions on February 22 and 2.72 millions on March 8, 1945.

Figure 4 illustrates the urinary 17-ketosteroids during the period before and after bilateral adrenalectomy. It should be recalled that bilateral orchiectomy had been performed 15 months before these studies. In spite of no hormonal medication between

TABLE I

SUBSTITUTION THERAPY FOR TOTAL ADRENALECTOMY*

0 minus 1 day		
D C A	5 cc. intramuscularly	10 A.M.
Adrenal cortex extract	5 cc. intramuscularly	6 P.M.
Adrenal cortex extract	5 cc. intramuscularly	10 P.M.
0 day		
Adrenal cortex extract	10 cc. intramuscularly	6 A.M.
Adrenalectomy		8 A.M.
D C A	5 mg. intramuscularly	9 A.M.
Adrenal cortex extract	20 cc. intravenously	9 A.M.
Plasma	500 cc. intravenously	9 A.M.
Dextrose 5% in water	500 cc. intravenously	9 A.M.
Adrenal cortex extract	10 cc. intramuscularly	Each hour until 8 P.M
Plasma	500 cc. intramuscularly	7 P.M.
Dextrose, 5% in water	500 cc. intramuscularly	7 P.M.
Adrenal cortex extract	5 cc. intramuscularly	Each hour
0 plus 1 day		
Adrenal cortex extract	5 cc. intramuscularly	Each hour until 8 A.M.
Plasma	500 cc. intramuscularly	8 A.M.
Dextrose, 5% in water	500 cc. intramuscularly	8 A.M.
D C A	5 mg. intramuscularly	8 A.M.
Adrenal cortex extract	5 cc. intramuscularly	Every second hour until 8 P.M.
Adrenal cortex extract	5 cc. intramuscularly	Every three hours until 8 A.M.
0 plus 2 days and 0 plus 3 days		
D C A	5 mg. intramuscularly	8 A.M.
Adrenal cortex extract	5 cc. intramuscularly	Every four hours
0 plus 4 days		
D C A	5 mg. intramuscularly	8 A.M.
Adrenal cortex extract	5 cc. intramuscularly	Every six hours
0 plus 5 days		
D C A	5 mg. intramuscularly	8 A.M.
Adrenal cortex extract	5 cc. intramuscularly	Every eight hours
0 plus 6 days		
D C A	5 mg. intramuscularly	8 A.M.
Adrenal cortex extract	5 cc. intramuscularly	Every twelve hours
0 plus 7 days		
D C A	5 mg. intramuscularly	8 A.M.
Adrenal cortex extract	5 cc. intramuscularly	8 A.M.
Succeeding days		
D C A	5 mg. intramuscularly	8 A.M.

* We are indebted to Professor George W. Thorn of Harvard University for suggesting this regimen; without his aid prolonged survival would hardly have been possible.

removal of the left and right adrenal glands these values are higher than before the removal of the left adrenal gland. This phenomenon was not observed previously. Following removal of the remaining adrenal gland, the level of urinary 17-ketosteroids, total, ketonic and *alpha* fractions, rapidly fell and remained low for 116 days, the duration of survival. These total values are higher than the actual amount of 17-ketosteroid present in that crude color as the result of impurities adds to the total color of the reaction. This is apparent from the ketonic fraction and from studies made in which the entire

urine for the last 49-day period was extracted. During this period there was excreted on average 1.0 mg. per day of ketonic 17-ketosteroid. Further purification by means of chromatographic absorption technic reduced this to less than 0.7 mg. per day. At least one crystalline substance has been isolated from this 49-day collection but as yet has not been identified.

Of interest in Case 4 was the continued excretion of estrogen in the urine after both the testes and adrenals were removed. This patient excreted about five International Units of estrogen per day, expressed in terms of *alpha* estradiol benzoate, for a period of 41 days following adrenalectomy. The source of this estrogen is speculative but may have been degradation of desoxycorticosterone acetate or adrenal cortical extract.

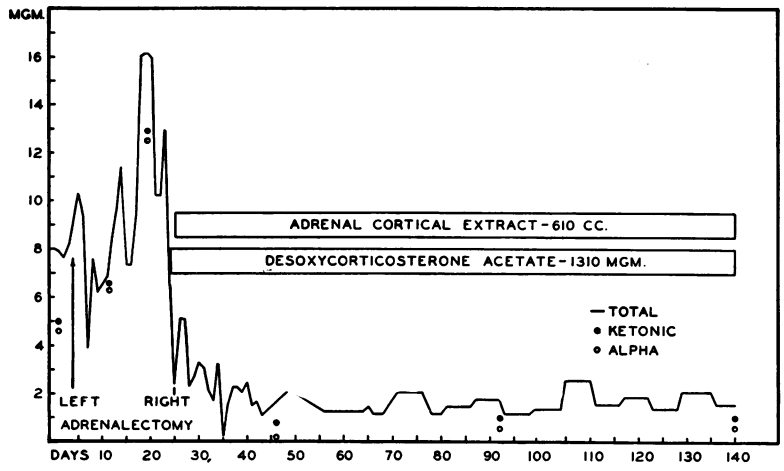

FIG. 4.—Decrease of alpha, ketonic and total urinary 17-ketosteroids following complete adrenalectomy with survival for 116 days, Case 4.

DISCUSSION.—In Cases 1 and 2 the substitution therapy was obviously inadequate since these men died 36 and 54 hours after complete adrenalectomy. The most striking effects of immediate surgical adrenal insufficiency are fever and hypotension, with death in shock; here fever of 38° to 40° C. arose within eight hours, and was maintained until death. The blood pressure remained at a normal level for 12 and 36 hours when it decreased to a shock level. The carbohydrate metabolism was not drastically affected since the level of blood glucose was not decreased and hypoglycemic convulsions were never seen. Lowering of serum sodium and elevation of serum potassium was not profound, and these levels were as much affected by removal of one adrenal as both. We are of the opinion that the large transfusions of plasma are of great aid in preserving life.

A significant effect following adrenalectomy was the rapid fall in urinary 17-ketosteroids, a fall which was persistent in the patient surviving 116 days. There has been previous, suggestive evidence that the testes and adrenals are the chief sources of the urinary 17-ketosteroids. Fraser, *et al.*,[11] giving total values of 14 and 9 mg. per 24 hours for the average excretion of 17-ketosteroids in the male and female, respectively, have postulated that 5 mg. comes from the testes and the remainder from the adrenal. Further evidence for this

concept is given by the markedly diminished or absent 17-ketosteroid excretion in the female with Addison's disease.[11, 12] Direct proof, however, such as was afforded by total absence of the testes and adrenals, has been lacking until the present study.

The rapidity of onset of pigmentation is of considerable interest. Sixteen days after complete adrenalectomy in Case 4 dark brown pigment was first observed in the lower eyelids, over the skin of the back and at the site of ad-

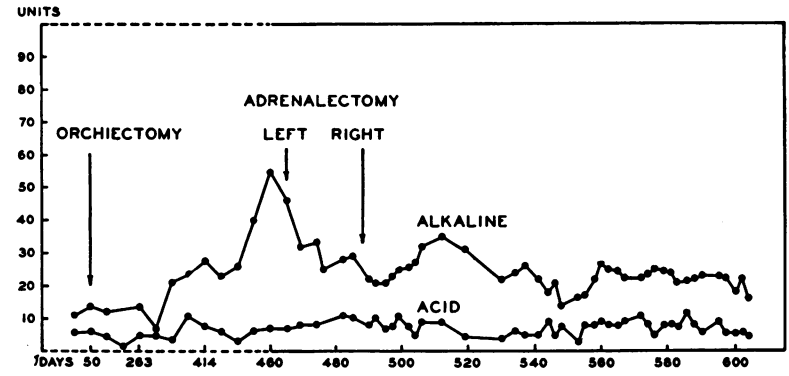

FIG. 5.—Rise in alkaline phosphatase 1 year after orchiectomy with a decrease after adrenalectomy although not to normal levels, Case 4. The phosphatase values are expressed in King and Armstrong units per 100 cubic centimeters of serum.

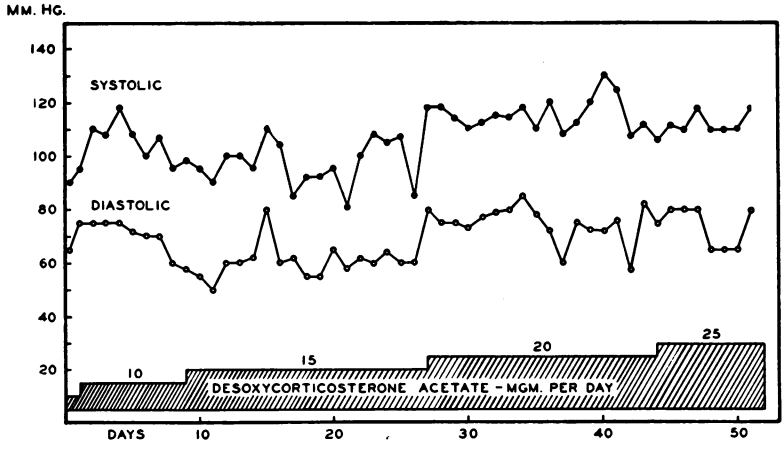

FIG. 6.—Failure to develop hypertension with increasing dosage of desoxycorticosterone acetate in an adrenalectomized man, Case 4.

hesive strapping. This coloration increased and appeared on the dorsum of the hands and on the external genitalia. The feet were uninvolved. The early appearance of this pigmentation was significant; pigmentation does not occur early in Addison's disease. The fact that the pubic and axillary hair became sparse was interesting.

The alkaline phosphatase of the serum decreased considerably after removal of both adrenals (Fig. 5) although not to normal values. This signifies a reduction in osteoplastic activity. The primary tumor also underwent a decrease in size and hardness as compared with the preadrenalectomy findings.

The effect of desoxycorticosterone acetate on blood pressure of the adrenalectomized man was of interest in that hypertension was not produced. Loeb,[13] Thorn,[14] and others,[15] have demonstrated the considerable rise in blood pressure occurring in Addison's disease as a result of overtreatment with DCA. In five of the six cases reported by McCullough and Ryan[16] hypertensive levels were reached with hormonal dosage of 10 mg. daily, or more. Clinton and Thorn[17] have found in normal subjects that a striking increase in plasma volume with retention of sodium and chloride followed the administration of 10 mg. of DCA daily.

Thorn, Koepf and Clinton[18] have described a syndrome characterized by excessive loss of salt and water resulting in collapse as a result of renal disease; these authors observed that adrenocortical homones were of no effect apparently because damage to the renal tubules rendered them unresponsive to these agents while permitting excessive loss of sodium and chloride. In Case 4 in the present paper, DCA was effective in maintaining blood pressure at normal values but hypertension was not achieved with amounts of 25 mg. daily (Fig. 6) even with addition of sodium chloride, 8 Gm. daily, to the diet; the maximum blood pressure of 130/70 was obtained with a daily DCA dosage of 20 mg. Renal damage of severe grade was eliminated from consideration in this case since phenolsulfonephthalein excretion, blood urea and urea clearance values were within the normal range. This case is, therefore, exceptional with respect to resistance to DCA, but the reason for the lack of response is not clear.

Advancing carcinomatosis in man with low androgenic activity calls for a reëxamination of the factors involved in prostatic cancer. Three factors may be discerned at the present time, namely, the testicular androgens; the extragonadal androgenic depot (which may now be stated to be the adrenal glands exclusively); and androgen-independence. In many patients widespread carcinomatosis undergoes involution, both extensive and prolonged, following excision of the testes; here clearly the tumor is androgen-dependent and the testes are producing a highly significant fraction of male hormone, In a very few cases estrogen is required after orchiectomy to control the neoplastic activity and the extragonadal depot may be postulated as a complicating androgenic factor.

The androgen-independent cancers are those in which antiandrogenic therapy fails. Following castration of normal males the prostate undergoes great reduction in size and there is a cessation of secretion; the epithelium of the normal prostate shrinks markedly in size *but the epithelial cells do not disappear.* Androgen is responsible for the development of the prostatic cells and alveoli, but having developed they acquire a value which permits survival

(in a vestigial condition) in the androgen-free state and, thus, are androgen-independent. Androgen-independence is not remarkable since it is physiologic; what is noteworthy is the necessity for many of the prostatic cancers to be furnished with androgen for their continued activity.

CONCLUSIONS

Abundant use of plasma transfusions seems to be of great importance in preventing circulatory collapse following adrenalectomy in man.

Inadequate therapy after adrenalectomy in man results in early hyperpyrexia and hypotension; the carbohydrate metabolism is not drastically disturbed. In the adrenalless man adequately treated with plasma, adrenal cortical extract and desoxycorticosterone acetate these effects were not observed but addisonian pigmentation occurred on the 16th postoperative day, and was progressive.

Complete adrenalectomy in castrate man results in a reduction of 17-ketosteroids to values less than two milligrams excreted in the urine daily; total, ketonic and *alpha* fractions are greatly diminished. Urinary androgens as measured by the comb-growth technic were absent. There is a continued excretion of small amounts of estrogen. In a man who survived complete adrenalectomy for 116 days there was a sustained reduction of alkaline phosphatase activity of the serum but the prostatic cancer progressed, although apparently at a retarded rate. DCA elevated blood pressure to normal levels but hypertension did not occur with massive doses.

The extragonadal androgenic depot in man is the adrenal.

Adrenalectomy is not a practical method of treatment of the failure-group of patients with prostatic cancer treated by antiandrogenic methods.

Three factors, whose presence and significance vary in the human prostatic cancers, may be stated: They are the testicular androgens; the extragonadal depot; and androgen-dependence or its opposite, -independence. It is not yet possible to define androgen-dependence or -independence in chemical terms.

BIBLIOGRAPHY

1 Huggins, C., and Hodges, C. V.: Cancer Research, 1, 293, 1941.
2 Huggins, C., Stevens, R. E., and Hodges, C. V.: Arch. Surg., 43, 209, 1941.
3 Price, D.: Am. J. Anat., 60, 79, 1936.
4 Spiegel, A.: Virchows Arch. f. path. Anat., 305, 367, 1939.
5 Wooley, G. W., and Little, C. C.: Cancer Research, 5, 211, 1945.
6 Moore, R. A., and Melchionna, R. H.: Am. J. Cancer, 30, 731, 1937.
7 Schlotthauer, C. F., and Millar, J. A. S.: J. Am. Vet. Med. Assoc., 99, 239, 1941.
8 Krause, C.: Zeits. Path., 41, 405, 1931.
9 Roth, L.: J. Am. Vet. Med. Assoc., 95, 232, 1939.
10 Engle, E. T., and Stout, A. P.: Am. J. Cancer, 39, 334, 1940.
11 Frazer, R. W., Forbes, A. P., Albright, F., Sulkowitch, H., and Reifenstein, E. C., Jr.: J. Clin. Endocrinol., 1, 234, 1941.
12 Friedgood, H. B.: The Chemistry and Physiology of Hormones. Washington, 1944. Amer. Assoc. for Advance. of Sci., p. 195.

[13] Ferrebee, J. W., Ragan, C., Atchley, D. W., and Loeb, R. F.: J. Am. Med. Assoc., **113**, 1725, 1939.
[14] Thorn, G. W., and Firor, W. M.: J. Am. Med. Assoc., **114**, 2517, 1940.
[15] Soffer, L. J., Engel, F. L., and Oppenheimer, B. S.: J. Am. Med. Assoc., **115**, 1860, 1940.
[16] McCullagh, E. P., and Ryan, E. J.: J. Am. Med. Assoc., **114**, 2530, 1940.
[17] Clinton, Jr., M., Thorn, G. W., Eisenberg, H., and Stein, K. E.: Endocrinol, **31**, 578, 1942.
[18] Thorn, G. W., Koepf, G. F., and Clinton, M.: New England J. Med., **231**, 76, 1944.
[19] Scott, W. W., and Vermeulen, C.: J. Clin. Endocrinol., **2**, 450, 1942.