when intraventricular pressure decreases(4). Although the concept of the heart drawing fluid in during diastole is old(5,6,7) the magnitude of the negative pressure which can build up in diastole indicates that this mechanism is more important in cardiac filling than has been considered in the past.

We wish to thank Dr. Noble O. Fowler and Dr. E. Ronald Duschesne for their assistance.

Following the preparation of this paper, a communication with Dr. Gerhard Brecher of Department of Physiology, University of Ohio indicates that he has also obtained negative intraventricular pressures in the dog.

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Effect of Steroid Administration on Excretion of α-Ketolic Steroids and Sulfur in Cats. (22786)

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In the course of treating cats with 9α-fluorohydrocortisone and Δ^1-cortisone (prednisone), the changes in the chromatographic pattern of urinary steroids were measured. Two principal changes from the normal were encountered. (a) The α-ketols which were excreted increased only slightly, but there were changes in the proportions of several steroid metabolites. (b) It was unexpectedly found that elemental sulfur was present in large amounts in the chloroform extracts of these urines and that this interfered with the determination of α-ketols by the blue tetrazolium (B.T.) method, since elemental sulfur was found to give a positive B.T. reaction. After the separation of sulfur and steroids a significant increase in the urinary excretion of sulfur-containing compounds during steroid treatment was observed.

Methods. 9α-Fluorohydrocortisone and Δ^1-cortisone were administered subcutaneously in daily doses of 10 and 50 mg respectively.† The animals were fed diets of fresh meat which were constant during control and treatment periods. After 5 days or more of treatment, all urine was collected for 4 to 7 day periods and pooled. The pooled urine was then incubated with β-glucuronidase at pH 4.5 for 48 hours, extracted with chloroform at pH 1.0, and the chloroform extract washed briefly with 0.1 N NaOH and water. The B.T.-reacting materials were measured as described by Touchstone and Hsu(1). After the determination of total B.T.-reacting substances the remainder of the extract was subjected to paper chromatography.

Results. Fig. 1 shows three representative chromatograms. It appears that the principal metabolites in the normal cat occur in the region where H_4B, allo-H_4B, H_4A and H_4DOC appear (see legend for key to abbreviations). During the administration of 9α-fluorohydrocortisone and Δ^1-cortisone peaks can be found in the regions of H_4F, H_4E, B, A, and H_4DOC. Δ^1-Cortisone also caused excretion of three metabolites which were not found during administration of 9α-fluorohydrocortisone, and it seems that a large portion

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† We are grateful to Merck and Co., and to E. R. Squibb and Sons for supplying these steroids.

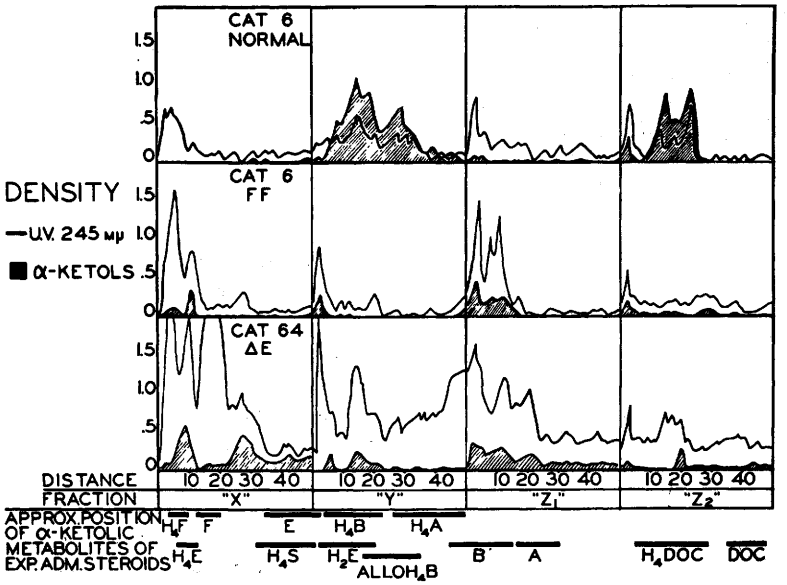

FIG. 1. Representative paper chromatograms of urinary steroid excretion in normal cat and in cats treated with 10 mg of 9α-fluorohydrocortisone (FF) daily and 50 mg of Δ^1-cortisone (ΔE) daily respectively. Chromatograms charted are based on one of eighteen ½ in. strips, each strip representing about 25% of daily steroid excretion(3). Unshaded area represents ultraviolet absorption at 245 mμ, using a Beckman spectrophotometer and special adapter for the chromatographic strips. Steroids having α,β-unsaturated ketone groupings in the A ring will be detected at this wave length, whereas compounds containing a saturated A ring (tetrahydro-forms) show no absorption. The solid silhouette represents α-ketolic compounds which reduce B.T. These were obtained by spraying strips with B.T. and measuring absorption at 600 mμ. No definite identification of the materials represented by these peaks has yet been made. On bottom of chart the approximate locations of the various metabolites in human urines chromatographed under similar conditions by Dohan, Touchstone and Richardson(3) have been drawn. H_4 = tetrahydrocortisone; H_2 = dihydrocortisone; F = hydrocortisone; E = cortisone; S = Reichstein's compound S (11-desoxy-17-hydroxycorticosterone); B = corticosterone; A = 11-dehydrocorticosterone; DOC = 11-desoxycorticosterone. Fraction "X" represents the compounds left on paper after 15-16 cc of effluent/½ in. strip had been collected in a toluene-propylene glycol system. Fraction "Y" represents rechromatography of effluent in the same system, collecting 3 cc of effluent. Fractions "Z_1" and "Z_2" both were chromatographed in methylcyclohexane-propylene glycol, collecting 15 and 5 cc of effluent respectively. Elemental sulfur, but no appreciable amount of α-ketolic steroids, was found in the last effluent "Z_2." Distance—each 10 units = 6.5 cm on paper.

of Δ^1-cortisone is excreted as non-α-ketolic substances. By these patterns, and from histologic sections of the adrenals of the cats(2) it can be inferred that both steroids depress adrenal function.

Determinations on the initial extracts of urine with one application of B.T. yielded surprisingly high values, equalling, or even exceeding, those of man. Erratic results also indicated that the color development was slow and incomplete. By applying B.T. twice, with water washing between applications, higher and more consistent values were found. A third application resulted in no further color development. Cortisone and other steroid standards gave the same results as before. Both the single and double applications yielded a significant increase in B.T.-reacting substances after the administration of Δ^1-cortisone or 9α-fluorohydrocortisone, to the extent that in a few instances the excretion in milligrams exceeded the amount of steroid given. Further investigation showed that the bulk of B.T.-reducing material was elemental sulfur. By the double application method standard solutions of sulfur in chloroform gave a curve conforming to Beer's Law, with a slope similar to that of the steroid standards.

Fig. 2 shows the daily excretion of B.T.-positive materials in cats during control peri-

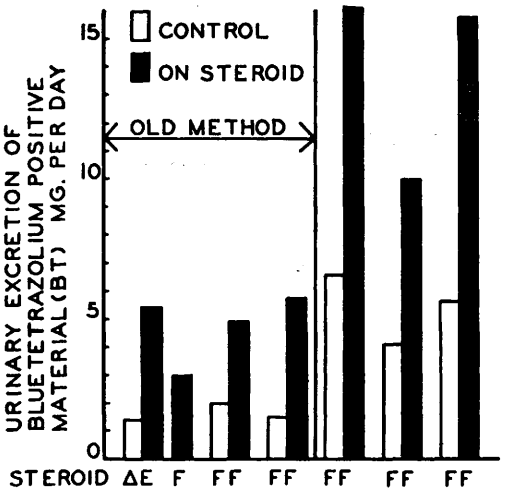

FIG. 2. Daily urinary excretion of blue tetrazolium-positive materials in cats during control periods and during steroid therapy. Old method: One application of blue tetrazolium. The 3 pairs of columns on the right show results after 2 applications of blue tetrazolium. One cat on 10 mg/day of hydrocortisone (F) showed slightly increased excretion. FF = 9α-fluorohydrocortisone.

ods and during steroid therapy. There is a consistent increase during therapy. Not charted are the amounts of B.T.-positive materials in the urine of one Houssay cat and three depancreatized animals, which were all within, or lower than, the normal range.

Fig. 3 shows the relative amounts of sulfur and steroids in extracts from which the sulfur has been separated by chromatography for 12 hours in the methylcyclohexane-propylene glycol system, by which method the less polar sulfur is found in the effluent and the α-ketolic steroids remain on the paper. The amount of steroid is small in comparison to the concentration of sulfur.

Having found that the usual extraction procedure yielded sulfur which interfered with the determination of steroids by B.T., we tried hydrolysis at pH 4.5 instead of pH 1 and found that no sulfur appeared in the extract. Evidently acid hydrolysis is an important determinant in liberating elemental sulfur.

Discussion. These questions now arise: What is the origin of the sulfur, and what is the significance of the increase in sulfur excretion during steroid administration?

Westall(4) reported that cats excrete in the urine large amounts of a peptide, which he called felinine. This compound has been tentatively found to consist of a combination of cysteine and isoamyl alcohol, combined in a thioester linkage. However, extraction of cysteine under the conditions used here failed to show any decomposition to sulfur. The diet does not seem to account for the increase in sulfur excretion, as the cats were fed the same amount of meat before and during therapy.

Negative nitrogen balance *per se,* as demonstrated in the Houssay animal and three depancreatized cats, does not appear to produce an increase in the sulfur excretion. Neither does the presence of glycosuria have an influence, as some steroid treated cats were aglycosuric during the collection period. We have observed that animals treated with steroids have no hair growth, and that the skin is thin and friable. In one cat, gentle handling caused the skin to rupture. There is also severe wasting of muscle, with profound muscle weakness. Both hair, epidermis and muscle are rich in sulfur-containing substances. It is possible that the steroids upset normal metabolism of certain sulfur-containing compounds in these tissues. However, by these methods we have not found sulfur in the urine of man during doses of prednisone up to 80 mg daily.

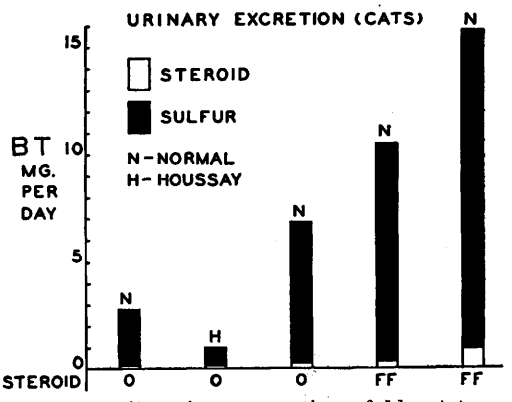

FIG. 3. Daily urinary excretion of blue tetrazolium-positive materials in cats. Sulfur and steroids have been separated. Note that normal untreated cats also excrete sulfur-containing compounds extractable as elemental sulfur. FF = 9α-fluorohydrocortisone.

Summary. 1. Administration of Δ^1-cortisone and 9α-fluorohydrocortisone changes normal urinary excretion of steroid metabolites, indicating adrenal depression or altered steroid metabolism. 2. Only a small proportion of administered 9α-fluorohydrocortisone is excreted as α-ketolic metabolites; Δ^1-cortisone also is converted to a great extent to non-α-ketolic elements. 3. Elemental sulfur has been found to give a positive reaction with blue tetrazolium. This interferes with the quantitative determination of α-ketols in extracts of cat urines. 4. The α-ketols and sulfur can be separated by chromatography and each determined quantitatively by the blue tetrazolium reaction, using a modified method. 5. Administration of Δ^1E and 9α-fluorohydrocortisone causes an increase in urinary excretion of sulfur-containing compounds in cats, which leads us to conclude that these steroids alter sulfur metabolism.

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Prostatic Neoplasms of the Wistar Rat Induced with 20 - Methylcholanthrene. (22787)

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Prostatic tumors have never been reported to occur spontaneously in the rat, in contrast to man(1) and dog(2,3). McCoy(4) examined nearly 100,000 wild rats finding 103 primary tumors, none of which originated in the prostate. Moore and Melchionna(5) were first to induce with 1:2 benzpyrene in lard squamous cell carcinoma and sarcoma of the anterior lobe of the rat prostate but they did not report metastases. Dunning, Curtis and Segaloff(6) produced metastasizing squamous cell carcinoma in rat prostate with methylcholanthrene but the designation of the prostatic lobe where this carcinoma was induced was not mentioned. Neither groups reported occurrence of adenocarcinoma of the rat prostate.

The purpose of this paper is to report the induction of three types of experimental neoplasms particularly adenocarcinomas in the ventral lobe of the prostate glands of Wistar rats using methylcholanthrene crystals.

Material and methods. 122 male inbred Wistar rats, 3 months of age, were kept in individual cages and given Purina Laboratory chow and water *ad libitum*. Under nembutal or ether anesthesia, the ventral prostate was exposed through a posterior midline incision. Using aseptic technic, a pocket was made in the ventral prostate, and crystals of 20-methylcholanthrene were carefully deposited. The pocket was then closed by suturing with surgical silk. Extreme care was taken to avoid scattering of methylcholanthrene. The technical success of the intraprostatic injections was evidenced by presence of a small yellow bleb. Following the operation, the rats were examined routinely for presence of palpable prostatic masses; biopsies were repeatedly performed to study the character of the growth. This method was used to establish the latent period of the tumor. 23 animals were excluded from evaluation because they died shortly after the operation. The remainder of operated rats, 99 in number, were kept until they showed signs of impending death. These were sacrificed and autopsied. Wherever possible, small fragments of prostatic tumors were removed and immedi-

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