plastin) → Thrombin. Solutions of prothrombin standing at 4°C go through most of this cycle, the prothrombin molecule alone possessing the peculiar properties that enables it to do this.

1. Mertz, E. T., Seegers, W. H., Smith, H. P., PROC. SOC. EXP. BIOL. AND MED., 1939, v41, 657.
2. Ware, A. G., Seegers, W. H., *J. Biol. Chem.*, 1948, v174, 565.
3. Seegers, W. H., Andrews, E. B., McClaughry, R. I., *Am. J. Physiol.*, 1951, v164, 722.
4. Seegers, W. H., *Record Chem. Progress*, 1952, v13, 143.
5. Seegers, W. H., Smith, H. P., *Am. J. Physiol.*, 1942, v137, 348.
6. Ware, A. G., Seegers, W. H., *Am. J. Clin. Path.*, 1949, v19, 471.
7. Alkjaersig, N., Seegers, W. H., *Am. J. Physiol.*, 1955, v183, 111.

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Plasma Ketosteroid in Adrenal-Tumor Bearing Mice.*†‡ (23832)

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Several investigators have shown that transplantable adrenal tumors in mice may produce steroid hormone. This has been demonstrated by anatomical changes in the hosts, such as the development of atrophic adrenal glands, mammary and uterine changes, or chemical studies either of urinary steroid or *in vitro* response of tumors to ACTH(1-5).

This report concerns the measurement of plasma ketosteroid in CDF_1 hybrid and Balb-c mice bearing a hormonally active, transplantable, adrenal tumor which arose spontaneously in a Balb-c mouse in the laboratory of Dr. H. B. Andervont of the National Cancer Institue.

Materials and methods. Determination of plasma 17-ketosteroids. Plasma ketosteroids were determined by a modification of the procedure of Gardner(6). Each determination represented a pool of plasma obtained from 4 mice. Mice were lightly anesthetized with ether and blood from the severed femoral vein was aspirated into a heparinized syringe. *Tumor inoculation and animal maintenance.* Two months old CDF_1 were injected subcutaneously with 0.1 ml of a 50% tumor-saline emulsion. Tumor-bearing animals were sacrificed on twenty-fifth day after inoculation for plasma steroid determination; control mice which had been maintained under similar conditions were sacrificed simultaneously. Mice were maintained at 24°C and fed Purina Chow.

Results. Tumor growth. After about 5 weeks of subcutaneous growth, the tumor was hemorrhagic and the animal died. Metastases were never observed in unoperated animals. The hosts developed atrophic adrenals; enlarged uteri were observed in females. Gross and microscopic details of tumor growth are being studied by Dr. T. B. Dunn of Nat. Cancer Inst.

The effect of tumor growth in male mice is illustrated in Fig. 1. After 25 days of tumor growth, the ketosteroid level is elevated with respect to the plasma of control mice. Gonadectomy-adrenalectomy decreased the plasma ketosteroid level of control mice to trace values but did not affect the elevated level found in the plasma of tumor-bearing animals. The tumor effected an increase in plasma ketosteroid roughly similar to that caused by administration of 1 mg of ACTH for 5 successive days. An unexpected finding was the presence of widespread gross metastases in such organs as lung, liver, and spleen in 11 of

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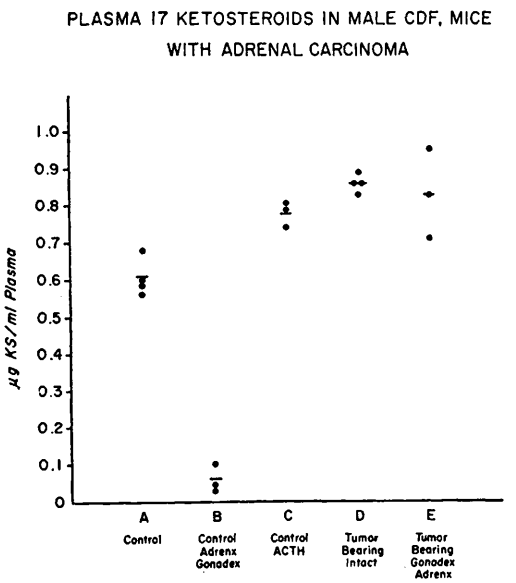

FIG. 1. Adrenalectomy-gonadectomy was performed on a control and tumor-bearing group 20 days after tumor inoculation in the latter group. ACTH was administered subcut., 1 mg daily, to a control group of mice for the last 5 days before sacrifice. Control and tumor-bearing animals were sacrificed after 25 days of tumor growth in the latter group. Each point represents a plasma ketosteroid determination on a pool from 4 mice.

12 adrenalectomized-gonadectomized mice. The development of metastases in these animals suggests that the tumor is not autonomous. This suggests that the tumor is hormonally controlled in unoperated tumor-bearing animals.

The effect of tumor growth on plasma ketosteroid in female mice is illustrated in Fig. 2. After 25 days of tumor growth, the plasma ketosteroid concentration is elevated with respect to the controls. Adrenalectomy decreased the plasma ketosteroid of control mice to trace values but did not affect the elevated level found in the plasma of tumor-bearing animals. Administration of ACTH to control mice caused an increase in plasma ketosteroids but no significant change in plasma concentration in tumor-bearing animals. Administration of Δ1-9α fluorohydrocortisone (Δ1-9FHC) to control mice decreased the plasma ketosteroid level. This powerful ACTH suppressant failed to affect the elevated plasma ketosteroid levels in tumor-bearing mice.

Distant metastases were present in 6/16 adrenalectomized mice. No metastases were found in any other group. Sham adrenalectomy was performed on 20 female tumor-bearing mice and plasma ketosteroid was within the range of unoperated tumor-bearing mice and no metastases were evident.

Discussion. Data have been presented to indicate that in the absence of the testis and adrenal, plasma ketosteroid concentrations fall to trace values. Adrenalectomized female mice bearing the transplanted adrenal tumor have elevated plasma ketosteroid concentrations and adrenalectomized gonadectomized male mice with tumor have elevated plasma levels. These data indicate that the tumor produces the excessive amounts of steroid. Steroid production is independent of ACTH since administration of ACTH, or Δ1-9α FHC produces no change in the plasma level.

Gallagher has demonstrated that some adrenal tumors in man are responsive to ACTH(7). Cohen *et al.* have studied adrenal tumors in mice and rats which respond *in vitro* to ACTH by increased steroid synthesis(5). Plasma ketosteroid studies should be helpful in distinguishing between responsive tumors of the type studied by these workers and tumors such as the type under present study which are not responsive to ACTH stimulation or suppression. The long term effects of ACTH, gonadotropin, and steroid hormones

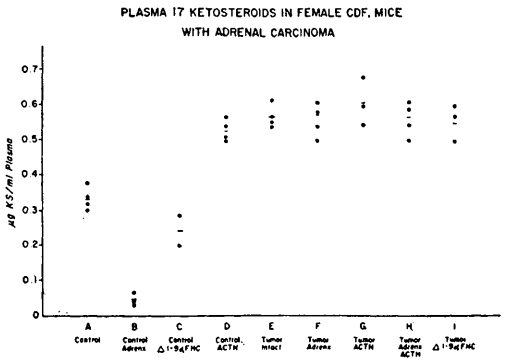

FIG. 2. Adrenalectomy was performed on groups of control and tumor-bearing mice after 20 days of tumor growth in the latter group. ACTH was administered where shown, 1 mg daily for the last 5 days before sacrifice. Δ1-9αFHC was administered where shown, 0.1 mg daily subcut. in oil, for the last 7 days before sacrifice. Control and tumor-bearing animals were sacrificed after 25 days of tumor growth in the latter group. Each point represents a plasma ketosteroid determination on a pool from 4 mice.

on the growth and behavior of this tumor are under investigation. These will be correlated with plasma ketosteroid and corticoid levels. If it could be demonstrated that chemotherapeutic agents affected growth and steroid biosynthetic capacity in a similar fashion in this tumor, the study of plasma steroid response to therapeutic agents may prove to be a useful screening technic.

Summary. A transplantable adrenal mouse carcinoma produced elevated plasma ketosteroid in the host. Non-tumor-bearing mice subjected to gonadectomy-adrenalectomy have negligible levels of plasma ketosteroid. Tumor-bearing mice have unchanged plasma levels following this procedure indicating steroid production by the tumor. ACTH and Δ1-9α fluorohydrocortisone have no influence on the plasma ketosteroid level of tumor-bearing animals. Distant metastases were found in adrenalectomized-gonadectomized males and adrenalectomized females, but not in animals not subjected to these procedures.

1. Dalton, A. J., Edwards, J. E., Andervont, H. B., Briggs, V. C., *J. Nat. Cancer Inst.*, 1943-44, v4, 329.

2. Kirschbaum, A., Franz, M., Williams, W. L., *Cancer Research,* 1946, v6, 707.

3. Gardner, W. U., *Cancer Research,* 1941, v1, 632.

4. Frantz, M. J., Kirschbaum, A., *ibid.,* 1949, v9, 257.

5. Cohen, A. I., Bloch, E., Celozzi, Evemarie, PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 304.

6. Poore, W., Hollander, V., *Endocrinology,* 1957, v61, 652.

7. Gallagher, T. F., *Cancer Research,* 1957, v17, 520.

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Inhibition of Glucose Uptake by Acidosis *in vitro*.* (23833)

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In diabetic acidosis there is a relative insensitivity to insulin(1). Chari(2) has concluded that a high concentration of acetoacetate is probably one of the factors causing a derangement of carbohydrate metabolism. The work of Guest *et al.*(3) shows however, that the excess hydrogen ion itself is an extremely important factor. They induced a simple ammonium chloride acidosis in dogs and demonstrated a reduced insulin sensitivity and a less favorable glucose tolerance. Guest attributes these effects to an inhibition of hexokinase at the lowered pH. Comparable experiments were conducted *in vitro* to determine whether extra-hepatic tissues were involved.

Method. Hemidiaphragms from rats weighing 150 $\pm$ 10 g were incubated at 38°C in Krebs-Ringer solutions buffered with bicarbonate-CO_2 at pH 7.4 and 6.8. Each solution contained 250 mg/L glucose and some insulin, and was equilibrated with 5% CO_2 in O_2. The glucose uptake of each hemidiaphragm was determined by measuring the disappearance of glucose from 2 ml of the nutrient solution in 1 hour. The glucose determinations were by the anthrone method(4).

Results. When the insulin concentration exceeded 0.08 i.u./ml the glucose uptake was the same at pH 7.4 as at 6.8, but with decreasing insulin concentrations the muscles at the lower pH took up significantly less glucose. With 0.008 i.u./ml insulin the difference was 25%, and it was 80% when no insulin was added to the nutrient medium. The absolute glucose uptake decreases with decreasing insulin concentrations so that the percentage differences become large. Table I lists both the percentage differences and the absolute differences in g/kg/hr at each insulin concentration. The latter are shown graphically in Fig. 1. From these results it may be concluded

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