

proteins, rabbit platelets could be labeled adequately *in vitro* with radioiodine. 2. Platelets thus labeled *in vitro* did not promote clot retraction and disappeared rapidly from the circulation on reinjection. 3. The probable reason for the failure of the tagged platelets to survive following reinjection is injury to the platelets, resulting from the separation and concentration technics. 4. Since separation of platelets from plasma proteins is essential for I^{131} labeling, this method does not appear adaptable to studies of the *in vivo*

metabolism of normal platelets.

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Glucocorticoids in Adrenocortical Carcinoma of Mice. (20903)

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Adrenocortical hyperplasia or carcinoma is observed in certain inbred strains of mice following early castration(1,2). In guinea pigs a nodular hyperplasia of the adrenal cortex following castration has been reported (3). Both the hyperplastic and the cancerous adrenals have been shown to secrete sex hormones, as evidenced by changes in the uterus and vagina(4), the prostate and seminal vesicles(5,3), the submaxillary gland and kidney(6) and the breast(4). Urinary secretion of estrogens has been studied in ovariectomized mice with adrenocortical tumors(7). Dickie and Woolley have studied the pituitary changes, especially with regard to the basophilic cells, in mice with postcastration adrenocortical cancers; they suggest that the syndrome in mice may well be an animal counterpart of Cushing's syndrome in man. Whereas no cell types in the mouse pituitaries were identified as exact counterparts of Crooke's changes, certain observed cell changes were considered in some respects similar to those described by Crooke, which in man are characteristic of Cushing's syndrome (8). Recent observations concerning the occurrences of Crooke's changes in individuals

treated with cortisone suggest that in Cushing's syndrome the Crooke's changes are the result of the excessive secretion of C_{11} - C_{17} oxygenated adrenal steroids(9,10). In the mouse, evidence has been presented heretofore only for sex steroid secretion by these adrenocortical carcinomas. It therefore appeared desirable to examine them for the presence of C_{11} oxygenated steroids.

Materials and methods. Adrenal cancers were induced in Minneapolis (a) in F_1 hybrids of AxCE mice and transplanted into hybrids as described elsewhere(11) (first transplant generation from 3 primary tumors), and (b) in CE mice, with transplantation into the same strain (pool second transplant generation from 4 primary tumors). The mice were shipped to Philadelphia, where the tumors were harvested when they had reached an appropriate size. The tumors were dissected out, weighed and homogenized in a Potter homogenizer. The homogenate was used for bioassay of "corticoids" by the mouse liver glycogen method of Venning(12). The tissue homogenate was mixed directly with the glucose-alcohol in some instances; in others an acetone extract was prepared, and the dried extract dissolved in alcohol and added to the glucose. In one experiment, 30

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TABLE I. Glucocorticoids in Adrenal Carcinoma of Mice. 6 experiments.

Origin of tumor	Wt of tumor (mg)	Equiv. of Compound A acetate		Assay
		In total tumor	Per 100 mg tumor	
AxCE		215		In fresh homogenate
"		240		<i>idem</i>
"	738	190	25	<i>idem</i>
"	1968.8	180	9.1	On acetone extract of homogenate
"	6000	264	4.4	<i>idem</i>
CE	5350	720	13.4	<i>idem</i> , incubated 2 hr*

* This procedure was performed because this experiment served as control for the incubation of tumor homogenate with desoxycorticosterone glucoside (see text).

mg of desoxycorticosterone glucoside was added to 5.35 g homogenate in Krebs buffer solution, and the mixture incubated in oxygen for 2 hours with constant shaking. This was followed by acetone extraction; the dried acetone extract was assayed as indicated above. A group of 47 control mice (hybrid white mice obtained from a local dealer) were sacrificed and the adrenals pooled and homogenized. Assay of the fresh homogenate was performed as indicated above for the tumors. Assay values were read against a standard dose-response curve with either compound A acetate or cortisone acetate.

Results. 1. Results of the assays of the tumors are summarized in Table I. Glucocorticoids were demonstrable in all instances.

2. An assay of the glucocorticoid content of normal mouse adrenals yielded 190 μ g equivalent of Compound A acetate in 206.8 mg adrenal tissue, representing 47 pairs of mouse adrenals; the concentration was 90 μ g per 100 mg adrenal tissue, or 4 μ g per pair of adrenals.

3. Incubation of 30 mg desoxycorticosterone glucoside with 5350 mg adrenal tumor homogenate for 2 hours failed to yield additional glucocorticoids; the assay value was 816 μ g equivalent of Compound A acetate, as against 720 μ g equivalent in the incubated homogenate without addition of DOCG.

Discussion. Glucocorticoids were demonstrable in the adrenocortical carcinomas. The values obtained may be considered as minimal values; in the larger tumors necrotic areas were detectable grossly and although as much as possible was excised prior to homogenization, very probably some residual necrotic material was included. In the normal ad-

renals, medulla and cortex could not be separated. The values for hormone concentration, expressed as μ g per 100 mg adrenal- or tumor tissue, are therefore of limited significance, but it would appear that the concentration in the normal adrenals is higher than in the tumor. On the other hand the amount of hormone present in an individual mouse is 50-100 times greater in tumor-bearing than in normal mice. In 2 of the tumor-bearing mice (Exp. 1 and 2) blood sugars were determined and were found to be 82 and 106 mg/100 ml, respectively. If the hormone content of the tumor reflects a comparable release of glucocorticoids into the circulation, it is perhaps remarkable that these mice were normoglycemic. Little is known regarding the sensitivity of mice to the diabetogenic action of cortical hormones; Furth has observed diabetes in mice with adrenocortical hyperplasia due to pituitary tumors(13). Perhaps the simultaneous secretion of sex steroids may counterbalance the effect of glucocorticoid excess in our experiments.

The fact that no conversion of 11-desoxycorticosterone to a glucocorticoid could be demonstrated, may suggest a different mode of synthesis in the tumors as compared to normal adrenal tissue. The latter, obtained from beef, can oxidize the steroid molecule at C₁₁(14). No comparable data for normal mouse adrenals are available. However, our incubation mixture was not reinforced with ATP, fumarate and magnesium ions, which increase conversion(14). In the absence of these agents, maximum conversion would not be expected, but the complete absence of conversion in the mouse adrenal tumor is probably significant.

The observation that these adrenocortical carcinomas which have been known to secrete sex hormones, also contain appreciable amounts of glucocorticoids, is of interest in view of the findings of Dickie and Woolley on the pituitaries of mice bearing adrenocortical cancers(8). The changes which they found in the pituitary basophils in these mice resembling those in human cases of Cushing's syndrome, may perhaps be correlated with glucocorticoid secretion by the cortical tumors rather than with the sex hormone secretion. In man, changes in the pituitary basophils, especially the hyaline changes described by Croke, are characteristic of Cushing's syndrome and have also been found in the pituitaries of individuals treated with large doses of cortisone(9,10). It is of interest too that an elevated level of formaldehydogenic steroids in the urine has been observed in some instances of adrenogenital syndrome due to adrenocortical carcinoma in women, although the clinical picture gave no evidence of excessive glucocorticoid effects. One may draw a tentative parallel to the mouse adrenocortical cancers reported in this paper.

Summary. Adrenocortical carcinomas, induced in mice by early castration, and transplanted to recipient mice, were assayed for glucocorticoids by the mouse liver glycogen method of Venning. Glucocorticoids were present in all tumors. The hormone content of

the tumors was compared with that of normal mouse adrenals and was found to be smaller on the basis of tissue weight. Incubation of tumor homogenate with desoxycorticosterone glucoside failed to yield increased glucogenic activity.

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Development of Experimental Exophthalmos in Scorbatic Guinea Pigs.* (20904)

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Since the publication of Wolbach's classic investigation of scurvy(1,2), it has been accepted that in the scorbatic state there is a deficiency in the formation of mesenchymal

intercellular substances. Production of both major elements of the connective tissue intercellular material, the fibrillar components as well as the amorphous ground substance, has been reported to be diminished or absent. There has been ample confirmation of the fact that collagen fibre formation in scurvy

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