which is somewhat less than that in rats treated for 5-7 days and considerably less than that seen in untreated infected animals. Hyperplasia of the spleen and lymph nodes was noted in both the treated and untreated animals and treatment with ACTH seemed to have no modifying effect.

In group B the rise in eosinophils (Fig. 1) is typical of that found in untreated trichinosis. The injections of physiological saline from the 14th to the 20th day, as might be expected, had no effect on the circulating eosinophils. The normal response of eosinophils to ACTH in a normal animal is illustrated in the curve of group C (Fig. 1). In group D the graph illustrates the normal day to day variation in eosinophils which showed no change with physiological saline injections.

Conclusions. 1. The marked eosinophilia due to an experimental Trichina infection was greatly depressed but not abolished when ACTH was administered for short intervals. 2. The effects of ACTH in animals with artificially induced trichinosis were exhibited by (a) alterations of the inflammatory response to encysted larvae, (b) alterations in the bone marrow, and (c) the changes in the level of circulating eosinophils. 3. Although modification of the host response to Trichina infestation was obtained with ACTH there was no objective evidence that the infected rats benefited as a result of this treatment.

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Steroid-3B-OL Dehydrogenase Activity and Androgen Production in Adrenal and Interstitial-Cell Tumors of Mice.* (21171)

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It has been demonstrated(1) that all normal endocrine tissues which produce non-benzenoid steroid hormones contain an enzyme which will oxidize certain Δ^5-unsaturated steroids with a 3-β alcohol group to the corresponding α,β-unsaturated Δ^4-3 ketone, the latter structure being characteristic of the more active C_{19} and C_{21} compounds formed by these tissues. This enzyme system has not been found in non-endocrine tissues nor in significant quantities in tissues producing only the benzenoid steroids. The present communication reports the presence of this enzyme, 3 β-ol dehydrogenase, in neoplastic tissues arising from the mouse adrenal cortex and testicular interstitial cells, and relates its concentration to the steroid hormone production of these tumors.

Materials and methods. Carcinomas producing sex steroids develop regularly in the adrenal cortices of CE mice several months

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after the animals have been castrated(2). Five such tumors were transplanted into genetically suitable intact recipients in which they grew and formed palpable tumors within 6 to 8 months. These tumors and subsequent transplant generations derived from them were employed in this study. In order to evaluate the production of androgenically active steroids by the grafted tumors, the recipient mice in most instances were castrated when the tumor transplants became palpable, usually from 1 to 3 months prior to the sacrifice of the animals.

Testicular interstitial cell tumors were produced in Bagg albino C mice in three ways: by subcutaneous implantation of a 3 mg fused pellet containing 10% diethylstilbestrol in cholesterol (C ♂ 166298), by rendering the mouse cryptorchid surgically and grafting a pair of ovaries from a young C strain female into the axillary tissue (C ♂ 164041 and C ♂ 166189) or by rendering the mouse cryptorchid surgically and placing it on a semisynthetic diet containing diethylstilbestrol in such an amount that the animal ingested approximately 0.5 γ of the hormone daily (C ♂ 157220). The resultant tumors, regardless of the method of production, were of similar histological pattern, and during the first few transplant generations were "dependent" in that they grew progressively in genetically suitable intact male mice only if these recipient animals were themselves estrogenized. Therefore, with the exception of one experiment, all tumors used in this study were grown in male mice that had been implanted with a fused pellet containing 10% stilbestrol, and in most instances the recipients also were castrated so that the production of androgenically active hormones by the tumors could be evaluated.‡

Androgen stimulation was detected by histological examination of the submaxillary salivary glands and of the seminal vesicles. The degree of stimulation was roughly quantified by weighing the seminal vesicles and adjoining coagulating glands, and in some experiments by weighing the left kidney. In order to simplify the tabulation of data, the degree of androgen stimulation was classified as "minimal" if the seminal vesicle-coagulating gland complex weighed less than 50 mg but the submaxillary glands histologically showed definite evidence of masculinization. The degree of stimulation was considered to be "moderate" in those animals in which the seminal vesicle-coagulating gland complex weight was greater than 50 but less than 150 mg (in such cases the seminal vesicles contained typical white secretion and the submaxillary glands were always of the male type), and as being "strong" when the seminal vesicle-coagulating gland complex weighed in excess of 150 mg.

For estimation of enzyme activity, the tumor transplants were dissected as free as possible from surrounding subcutaneous connective tissue. Histological examination showed that the adrenal cortical carcinomas grew as solidly cellular tumors with very little connective tissue stroma, and grew to fair size with relatively little necrosis of tumor cells. Similarly the testicular tumor transplants contained little supporting connective tissue but most frequently contained cystic spaces filled with sero-sanguinous fluid. The testicular tumors, therefore, were sliced open prior to homogenization in order to release as much of this fluid as possible.

The tumor tissue was then weighed and homogenized in a known volume of the serum-buffer mixture used for incubation. Where high activity was anticipated, the ratio of tissue to total volume was 10 mg per ml; otherwise, the concentration was 15 mg per ml. For each tumor duplicate 125 ml flasks with each of 2 volumes of homogenate (1 ml and 3 ml) were prepared. To each flask, 2 μmols Δ^5-pregnen-3β-ol-20-one were added and the total volume made up to 25 ml with the serum-buffer mixture. This consisted of equal parts of bovine blood serum and Krebs phosphate buffer pH 7.4, to which was added 0.04 M nicotinamide and 0.4 mM diphospho-pyridine nucleotide. The flasks were gassed with oxygen and incubated for 1 hour at 37 degrees. The protein was then denatured by boiling and the mixture was extracted with ethyl ether. Extraction and purification were carried out as

‡ This degree of estrogenization prevents the development of androgen-producing adrenal adenomas which regularly occur in this strain of mouse following castration alone.

TABLE I. Comparison of 3 β-ol Dehydrogenase Activity and Androgen Production in Various Transplant Generations of 5 Adrenal Cortical Carcinomas Arising in Castrate CE Mice. Androgen production by 4 of the tumor lines could not be estimated during the first transplant generation since the recipients had not been castrated prior to sacrifice. Where more than one value is given for enzyme activity, several tumors of the designated transplant generation were analyzed independently. Enzyme activity is expressed as μmols of α, β unsaturated steroid produced per g of tumor per hr of incubation.

	CE ♀ 41	CE ♀ 74	CE ♂ 65	CE ♀ 63	CE ♂ 04
Original donor:					
Androgen stim.	Strong	Moderate	Moderate	Minimal	None
1st transplant:					
Androgen stim.	—	—	"	—	—
Enzyme act.	19.0	9.3-10.0	9.6, 13.5	5.6-10.0	.0
2nd transplant:					
Androgen stim.	Strong		None	Minimal	None
Enzyme act.	27.3		—	7.1	.0, .0
3rd transplant:					
Androgen stim.			None	Minimal	None
Enzyme act.			.0	—	.0-1.7
5th transplant:					
Androgen stim.				Very minimal	None
Enzyme act.				.6-1.4	.4-.6

previously described(3). Conversion of the substrate to an α,β-unsaturated ketone was estimated spectrophotometrically by the height of the absorption peak at 240 mμ.

Results. Adrenal cortical carcinomas. The studies of 3 β-ol dehydrogenase activity in the transplanted adrenal cortical carcinomas are summarized in Table I. Definite activity was evident in 4 of the 5 tumor lines during the first 2 transfer generations, and the concentration of enzyme appeared to parallel roughly the degree to which the secondary male characteristics had been developed as a result of androgenic stimulation. No appreciable concentration of the enzyme was found in the fifth tumor line, and no masculinization of the tumor-bearing mice was evident. Furthermore, one tumor (CE ♂ 65) was moderately masculinizing in the first transplant generation and the enzyme was present in modest concentration whereas in the third transfer generation when the tumor no longer had a masculinizing influence the enzyme was no longer detectable.

Since 3 β-ol dehydrogenase is probably also involved in the synthesis of the active C_{21} steroids, the presence of active glucocorticoids in certain of these tumors is of interest. Tumors of the first transplant generation of CE ♂ 65 and of the second generation of CE ♀ 41, CE ♀ 63, and CE ♂ 04 were pooled for assay, and significant amounts of glucocorticoid activity were detected. When a later transplant generation of CE ♂ 04 tumor which had no appreciable 3 β-ol dehydrogenase activity was assayed alone, no glucocorticoid activity was demonstrable by the Venning procedure(4).

In these adrenal cortical carcinomas it would appear that the concentration of 3 β-ol dehydrogenase roughly parallels the amount of androgen being produced, and that when the tumors contain this enzyme they may also be producing active glucocorticoids. The concentration of the enzyme in the tumors studied, however, was well below that in normal adrenals in CE ♂ mice for on 2 separate assays in which homogenates of pooled whole adrenals were used the amount of steroid oxidized was found to be 150 and 156 μmol/g/hr.

Testicular interstitial cell tumors. All of the testicular interstitial cell tumors assayed were found to exhibit 3 β-ol dehydrogenase activity, Table II. Within any single transfer line of tumor the concentration of enzyme appeared to be moderately constant but seemed to bear no relationship to the production of androgenically active hormone by the individual transplants. The production of androgens by the various transplants differed greatly even when derived from the same primary tumor. This was particularly true in the

TABLE II. Comparison of 3 β-ol Dehydrogenase Activity and Androgen Production in Various Transplant Generations of 4 Testicular Interstitial Cell Tumors Induced in Bagg Albino C Mice. Each enzyme determination was carried out on a single transplanted tumor and comparison is made to the degree of masculinization evident in the individual recipients. All recipients had been implanted with a fused stilbestrol-cholesterol pellet and, where androgen production by the tumor was estimated, the recipients had been castrated.

Original tumor	Transplant generation	Masculinization of recipient	Enzyme activity, μmol/g/hr
C ♂ 164041	1	Strong	27.2
	1	Very minimal	47.4
	2	Minimal	28.6
	3	Strong	29.0
	3	"	33.2
	3	"	29.8
C ♂ 166189	1	None	19.3
	2	Questionable	19.3
	2	None	11.2
	2	Questionable	17.1
	3	Moderate	25.8
	3	"	26.5
	3	Minimal	27.5
C ♂ 166298	3	Strong	10.0
C ♂ 157220 (Line A)	4	Strong	17.2
	8	—	37.6
	8	—	18.4
	9	Strong	18.3
	9	Moderate	15.0
C ♂ 157220 (Line B)	3	None	44.6
	4	"	57.5
	4	"	49.2
	4	"	49.0
	6	—	27.1
	6	—	27.5
C ♂ 157220 (Line C)	3	Strong	7.8
	4	Minimal	22.3

earlier transplant generations studied and in the transfer lines originating from C ♂ 157220 where transfer Line B was maintained distinct from Lines A and C from the time of explantation from the primary tumor. Such variation most probably arose as the result of selection, since most primary testicular interstitial cell tumors vary considerably in histology from area to area, and the histology and function of the explants might be expected to vary depending upon the particular fragment of donor tumor inoculated. Such variation would be expected to decrease as successive transfers are carried out.

Although there appeared to be no obvious correlation between the production of active androgens and the concentration of 3 β-ol dehydrogenase in this group of tumors, it was felt desirable to determine whether variations in hormone production among individual tumors of a single generation of a given transfer line might be accompanied by significant alterations in enzyme concentration. For this study the ninth transplant generation of tumor C ♂ 157220 Line A was employed. The results of this study are summarized in Table III. It can be seen that the concentration of 3 β-ol dehydrogenase was essentially the same whether the tumors were not producing detectable androgen, *e.g.* in intact female animals, or were producing more than the testicular interstitial cells normally do in this strain of mouse, *e.g.* in castrate male animals implanted with 10% stilbestrol pellets and injected daily for the 3 weeks prior to sacrifice with 0.1 mg of horse pituitary gonadotropin (Armour's No. 317-115).§ Even in this latter case the production of androgen per tumor cell is probably much lower than in the normal testicular interstitial cells, the much greater mass accounting for the higher total output.

Accurate comparison of either the androgen production or the concentration of 3 β-ol dehydrogenase in testicular interstitial cell tumors with that in normal interstitial cells is impossible since interstitial cells make up such a small part of the normal testis. Incubation of homogenates of normal mouse testes produce only about 4 μmols of α,β-unsaturated steroid per g of testis per hr of incubation, an amount well below that produced by most of the tumor homogenates studied. Since, however, interstitial cells constitute probably only 1-5% of the weight of the normal testis it seems probable that both androgen production and 3 β-ol dehydrogenase concentration in the normal interstitial cells considerably exceeds that found in the tumors.

Discussion. These studies of 3 β-ol dehydrogenase activity in neoplastic tissues aris-

§ This preparation of horse pituitary gonadotropin contains considerable FSH activity and is approximately 5 times as active with respect to LH as is Armour's sheep pituitary LH preparation No. 227-80. We wish to thank Dr. Sanford L. Steelman of the Armour Laboratories for supplying this hormone preparation.

TABLE III. Comparison of 3 β-ol Dehydrogenase Activity and Androgen Production in Interstitial Cell Tumors of the Ninth Transplant Generation of C ♂ 157220 Line A under Various Experimental Conditions. Enzyme activity was determined on pooled tumors from number of animals indicated in second column, and degree of androgen production was evaluated on the basis of the criteria listed in columns 3, 4 and 5.

Type of recipient	No. of animals	Histology of submax. gl.	Lt. kidney wt (mg)	Seminal ves. wt (mg)	Enzyme activity in tumors, μmol/g/hr
Castrate males	2	♂	204, 187	74, 127	15.0
10% stilb.	3	"	314, 228, 299	205, 249, 264	18.3
Castrate males	2	"	320, 317	285, 316	14.5
10% stilb. Mixed-gonado.	2	"	331, 325	312, 346	15.8
Intact females	3	♀	126, 141, 148	—	14.4
	2	"	161, 168	—	14.6
Castrate males 10% stilb.	10	"	164-205 avg 182	14-49 (Squamous met.)	No tumor
Intact females	11	"	120-165 avg 146	—	" "
Intact males	25	♂	185-304 avg 246	187-323 avg 238	" "

ing from the adrenal cortex and from testicular interstitial cells of the mouse lend themselves to interpretation by recent theories of steroid hormone synthesis in normal tissues. The investigations of Hechter *et al.*(5) indicate that a sequential series of reactions occurs in the adrenal cortex that converts Δ^5-pregnen-3β-ol-20-one to the adrenocortical hormones. The first reaction is that catalyzed by 3 β-ol dehydrogenase resulting in the formation of the α,β-unsaturated Δ^4-3 ketone following which some molecules are oxidized first at C-17 while others are first oxidized at the C-21 position after which 17-hydroxylation cannot take place. They also demonstrated that ACTH acted at some step in synthesis prior to the formation of pregnenolone. Slaunwhite and Samuels(6) have shown that testicular interstitial cells are capable of forming testosterone from pregnenolone by the formation of the α,β-unsaturated Δ^4-3 ketone, followed by hydroxylation at C-17, and then by the splitting off of the side chain at C-17 to yield Δ^4-androstene-3, 17-dione which in turn is reduced to testosterone. Studies of 17 ketosteroid excretion after the administration of various C_{21} steroids indicate that hydroxylation at C-17 is apparently essential for the formation of C_{19} steroids from such compounds(7,8).

The positive correlation between the 3 β-ol dehydrogenase activity and the production of androgens by the adrenal cortical carcinomas studied would indicate that the step catalyzed by this enzyme might well be the limiting one in the synthesis of these steroids by the tumors. According to the postulated sequence just outlined, if this enzyme were the limiting factor for the formation of androgens it should also be limiting in the synthesis of the metabolically active corticoids since the formation of the α,β-unsaturated Δ^4-3 ketone is a step common to both. This would also mean that neither androgens nor corticoids could be formed if 3 β-ol dehydrogenase were absent. This seems to be borne out in the instances where both functions were measured. In the one transplant line of adrenal tumor which lacked the enzyme there was no evidence of androgen production and no glucocorticoid activity could be demonstrated in its extracts: on the other hand 3 of the 4 tumors whose pooled extracts possessed glucocorticoid activity also were producing androgenic compounds and the presence of the enzyme was demonstrated.

The fact that the single adrenal tumor line that did not contain 3 β-ol dehydrogenase had marked estrogenic activity also fits with observations made on normal tissue and in normal animals. Samuels *et al.*(1) did not find 3 β-ol dehydrogenase activity in the follicles

dissected with their membranes from cattle ovaries, even though corpora lutea from the same ovaries were quite active. Heard *et al.* (9) obtained conversion of isotopic cholesterol to neutral ketonic steroids in the mare, but found no conversion to estrogens. Furthermore when isotopic acetate was administered, the isotope was found in the alicyclic rings of estrone, equilen and equilenin but not in the aromatic portion. Thus, the evidence at present does not support the previous hypothesis that the estrogens are formed by further unsaturation of the neutral steroids.

In the case of the interstitial cell tumors, on the other hand, it is obvious that the step which limits the production of androgenically active compounds by the tumor tissue is not the oxidation of the 3 β alcohol group of ring A. Several tumors that were not producing detectable amounts of androgen exhibited relatively high 3 β-ol dehydrogenase activity while several very masculinizing tumors contained significantly lesser amounts of this enzyme. No tumor was observed, however, that was androgenically active and yet lacked the enzyme. Whether the deficiency in the non-androgen producing tumors occurs at some enzyme-catalyzed step prior to the formation of pregnenolone, resulting in a lack of substrate for 3 β-ol dehydrogenase, or occurs after α,β-unsaturation of the molecule, presumably at some step involved either in 17 hydroxylation or in the splitting of the C-17 side chain, is as yet unknown.

Data obtained in the course of these studies also would seem to bear in a negative manner upon the question of where pituitary LH exerts its principal effect in the chain of events leading to the production of active androgens. Considerable unpublished data, as well as the one study presented here (Table III), indicate that the production of androgens by certain transplanted interstitial cell tumors is, at least in part, under the influence of LH. Alterations in the production of androgens by the one tumor studied, however, was not accompanied by changes in 3 β-ol dehydrogenase activity. This would suggest that LH does not exert its effect primarily by altering the activity of this enzyme. Probably, like ACTH, it exerts its effect at some point rather earlier in the synthesis of the active hormones.

The fact that the 3 β-ol dehydrogenase concentration of the adrenal cortical carcinomas was well below that of normal adrenal cortices is in accord with the general observation that the development of "the malignant change" is usually, if not always, associated with a decrease in specific normal cellular functions resulting from a reduced synthesis of the proteins specialized for such functions. It is obvious in the case of these adrenal tumors that the extent to which the reduction in 3 β-ol dehydrogenase synthesis occurred in association with the development of malignancy varied considerably from tumor to tumor, and that further reductions tended to occur with successive transplantations. Similarly, reductions in 3 β-ol dehydrogenase synthesis probably also occurred during the neoplastic transformation of the testicular interstitial cells, but in this instance greater deficiencies in other enzymes associated with androgen production apparently occurred since in the resultant tumors 3 β-ol dehydrogenase was not the limiting factor in androgen synthesis. Considerable heterogeneity of androgen production was evident in different transplanted tumors originally derived from the same primary tumor. Since this was particularly evident in the early transplant generations, it is most likely explained on the basis of cell selection during transplantation. With further transplantation of any single tumor line, the differences in histological appearance and in androgen production by individual tumors lessened considerably indicating that the fundamental alterations that had occurred were heritable in nature.

Summary. Steroid-3 β-ol dehydrogenase activity was measured in successive transplant generations of experimentally produced neoplasms of the adrenal cortex and testicular interstitial cells of the mouse. Different transplants varied widely in both their masculinizing effect and enzyme activity. In the adrenal tumors the concentration of this enzyme roughly paralleled the masculinizing effect of the transplant, but this was not true in the interstitial cell tumors. Androgenic activity however, was never observed in the absence of measurable 3 β-ol dehydrogenase.

When compared with the sequence of reactions described in normal adrenal cortical and testicular interstitial cells the results are interpreted as indicating that the reaction catalyzed by 3 β-ol dehydrogenase may be the limiting step in androgen synthesis by the adrenal tumors but is not in the case of the testicular interstitial cell tumors.

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Stimulatory Effect of Carbohydrates on Aspartic Acid Deaminase Activity of *Bacterium cadaveris.** (21172)

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Two contrasting effects of glucose have been reported with respect to the aspartic acid deaminase system. The presence of this sugar during growth results in reduction of enzyme activity(1,2), whereas its addition to washed bacterial suspensions results in stimulation of deaminase activity(3). Presumptive evidence for the coenzymatic nature of material resulting from the acid degradation of glucose has been presented recently(4).

The present paper is concerned with the nature of the stimulatory effects of various carbohydrates on the aspartate deaminase system in resting bacterial suspensions, vacuum dried preparations, and cell-free enzyme fractions prepared by sonic vibrations.

Materials and methods. The organism studied was *Bacterium cadaveris* (Gale) cultured at 30°C for 16-18 hours in a medium composed of 1% yeast extract, 1% casitone, and 0.5% K_2HPO_4, adjusted to pH 7. The cells were harvested by centrifugation and washed once with distilled water. Resting bacterial suspensions were prepared by resuspending the washed cells in distilled water to give a cell concentration of 0.3 to 0.5 mg of bacterial nitrogen per ml. Phosphate aging was performed by the method of Lichstein(5) with the cell concentration adjusted to 0.5 to 1.0 mg bacterial nitrogen per ml. Dried cells were prepared by placing washed cell pastes over Drierite *in vacuo* for 24-96 hours; cell-free fractions by treating washed cells in a 9 KC Raytheon sonic oscillator for 10-15 minutes and collecting the supernatant fluid after centrifugation. The deamination experiments were performed in phosphate buffer at 37°C, using adequate controls without added aspartic acid. The reaction was terminated by the addition of 0.2 ml of 25% trichloroacetic acid, the tubes centrifuged and an aliquot of the supernatant fluid removed for ammonia determination by Nesslerization. Color was measured in a Klett-Summerson photoelectric colorimeter using a blue filter (400-465 mμ).

The carbohydrates tested for stimulating activity were glucose, acid degraded glucose

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