tivity per 100 g of tissue, the per cent changes reflect increases in activity which cannot be explained solely on the basis of hyperplasia of the gland.

Summary. Examination of 10 different inbred strains and 2 hybrids of mice indicated that the normal levels of amylase activity vary from one strain to another. Further, the activity of the enzyme in the salivary gland tissue is markedly altered by gonadectomy. The effect of such endocrinological imbalancing varies with each strain. In some strains enzyme activity remains unchanged. In some, activity increases while in others it decreases. The pattern of change was not always the same for females as for males of the same strain. In other words, we are dealing with an enzyme system which is related to the genotype, but which may be influenced in an undetermined fashion by endocrine manipulation.

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Influence of the Jensen Sarcoma, Walker 256 Carcinoma, and Endotoxin on Plasma- and Hepatic-Bound Carbohydrates In the Rat. (32419)

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We have previously reported(1) that concentrations of serum-bound carbohydrates were not elevated in animals bearing intramuscular transplants of the Jensen sarcoma and Walker 256 carcinoma. This was contrary to the general findings in tumor hosts (2). When a bacterial infection was present in the tumor or a bacterial endotoxin was injected into normal animals, however, the serum-bound carbohydrate concentration was elevated. These studies have now been extended by determining the extent of glucosamine-C^{14} incorporation into serum and hepatic proteins of control, tumor-bearing, and endotoxin-treated rats.

Materials and methods. The bacteria-free tumors(1) were carried as intramuscular transplants in the *rectus femoris* muscle of female Holtzman rats for 7 days. Control and experimental rats were selected of the same age and lot and were fed *ad libitum*. *E. coli* endotoxin (Difco), suspended to contain 100 μg/1.0 ml, was injected intraperitoneally 24 hours prior to experimentation. Glucosamine-1-C^{14} (50 μc/mg, Isotopes, Inc.) at a level of 5.0 μc/0.2 ml was injected into the femoral vein 30, 60, and 120 minutes prior to sacrifice. The animals were anesthetized with Na seconal and bled by cardiac puncture. One aliquot of blood (2.5 ml) was mixed with an equal volume of saline containing 0.01 M EDTA, and the mixture centrifuged at 2500 rpm in a clinical centrifuge for 20 minutes to obtain a red blood cell volume and a plasma fraction. The percent plasma volume of whole blood was calculated from these values. The diluted plasma was fractionated by precipitation with TCA, defatted, and dried using the

methods described by Robinson *et al*(3). The residues were dissolved in 5.0 ml of 0.2 N NaOH with warming. Aliquots were taken for protein and C^{14} assays and for hexosamine isolation. Hexosamine was isolated, after acid hydrolysis, by the Dowex 50 column technique and assayed for hexosamine(3) and for C^{14} activity. Another aliquot of whole blood was allowed to clot at 4°C overnight and the serum assayed for bound hexose and hexosamine by methods of Winzler(4). All radiometric assays were made in a windowless gas flow (Q gas) counter. Protein was determined by the method of Lowry *et al*(5) using bovine albumin as standard.

Livers were removed from the animal and perfused with cold 0.25 M sucrose until free of blood, and the tumors were removed intact. These were then homogenized in 0.25 M sucrose and the cell fractions obtained by the ultracentrifugal method described by Robinson *et al*(3). Aliquots of the homogenate and each subcellular fraction were mixed with an equal volume of 10% TCA, and the residue treated as described for plasma.

Results. Systemic values. Table I shows some systemic values for the 4 groups of animals. The typical serum hypoproteinemia and an increased serum volume were observed in the tumor hosts. These animals exhibited no elevation in the concentration of serum-bound carbohydrate or hexosamine whereas there was an increase in the endotoxin-treated animals.

Plasma glucosamine-C^{14} incorporation. Fig. 1 shows an increased specific activity of the glucosamine-C^{14} incorporated into the plasma protein of the tumor hosts and endotoxin-treated animals after 120 minutes.

Hepatic glucosamine-C^{14}. Although there were no apparent differences in the rates of glucosamine-C^{14} incorporation into TCA-insoluble hepatic proteins of control and tumor host between 30 and 120 minutes, the magnitude of the specific activities differed (Fig. 2). The rate of incorporation of the

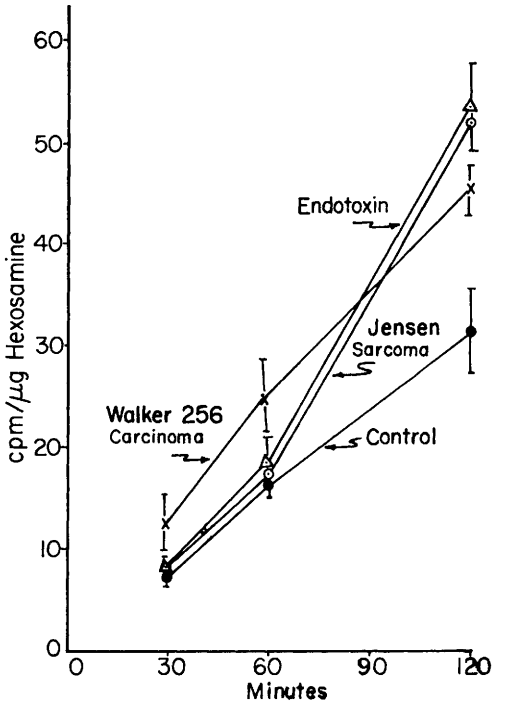

FIG. 1. Specific activity of glucosamine-C^{14} incorporated into plasma proteins. Control, —.—; Jensen sarcoma, — ⊙ —; Walker 256 carcinoma, — × —; endotoxin, — △ —. Verticle bars represent standard error.

TABLE I. Systemic Effect of Jensen Sarcoma, Walker 256 Carcinoma, and Endotoxin.*

	Control	Jensen host	Walker host	Endotoxin treated
Body or carcass wt (g)	191.3 ±3.8 (24)†	196.7 ±2.5 (22)	220.8 ±4.4 (12)	180.0 ±2.6 (22)
Liver wt (g)	8.11 ± .14 (23)	9.95 ± .21 (17)	11.38 ± .16 (12)	7.88 ± .12 (22)
Tumor wt (g)	—	16.91 ± .94 (17)	25.54 ±1.66 (12)	—
Serum:				
% Blood vol‡	46.9 ±2.9 (15)	68.3 ±1.5§ (17)	63.9 ±1.4§ (9)	53.8 ±2.6 (16)
Bound CHO (mg%)	108.1 ±3.4 (15)	90.5 ±2.6 (12)	115.8 ±4.0 (11)	133.6 ±6.4§ (11)
Hexosamine (mg%)	140.6 ±5.2 (11)	131.2 ±1.5 (11)	131.0 ±3.4 (11)	172.7 ±3.8§ (7)
Protein (g%)	7.31 ± .05 (12)	5.12 ± .15§ (7)	4.47 ± .02§(11)	7.73 ± .04§ (8)

* Mean values ± standard error.
† No. of animals.
‡ Standard hematocrit procedure gave 53.5% for control and 65.6% for tumor-host plasma volumes.
§ $P < 0.02$.

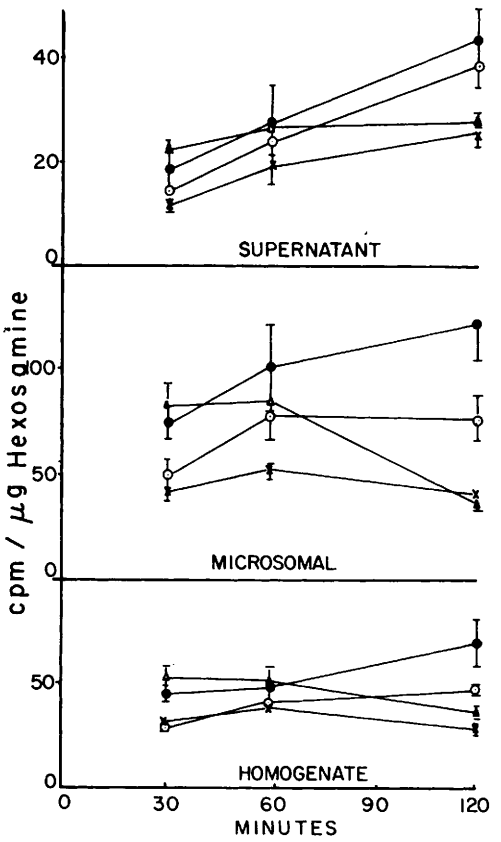

FIG. 2. Specific activity of glucosamine-C^{14} incorporated into hepatic proteins. See Fig. 1 for symbols.

endotoxin-treated animals, however, showed a peak incorporation at 60 minutes and then diminished. The variation in magnitude of the specific activities of the control and tumor host suggested a hepatic endogenous pool, which diluted the glucosamine-C^{14}, in the tumor host. Analysis of the liver homogenates revealed 3.11 ± 0.13, 4.38 ± 0.10, and 3.87 ± 0.18 μg hexosamine/mg protein in the control, Jensen sarcoma host, and endotoxin-treated animals, respectively. No significant change occurred in this pool size of the respective tissues during the time period studied.

TCA-soluble fractions. To determine whether our observation was further influenced by the incomplete precipitation by TCA of a highly labeled fraction in the tumor host, the TCA-soluble fractions were dialyzed for 48 hours against 4 changes of H_2O at 4°C. The dialyzed portion was then assayed for C^{14} and protein. No detectable protein (<0.05%) was observed in the dialyzed plasma or tumor TCA-soluble fractions, but 0.21, 0.18, and 0.23% of the total liver protein was nondialyzable in the control, tumor host, and endotoxin-treated animals, respectively. The C^{14} values are shown in Table II. An increase with time was noted in the control and tumor-host values, but there were no differences between the 2 groups. The endotoxin-treated animals, however, showed that 5.5% of the liver and 61.6% of the plasma TCA-soluble C^{14} were nondialyzable after 120 minutes. In the tumor homogenate, 5.0% of the total C^{14} was nondialyzable after 120 minutes; no detectable protein was found.

Tumor incorporation of glucosamine-C^{14}. The microsomal and supernatant fractions showed continued glucosamine-C^{14} incorporation up to 120 minutes, with the microsomal fraction having the greater specific activity (Fig. 3).

Discussion. Although serum concentrations did not reflect it, these data suggest that the synthesis of serum glycoprotein in animals hosting 7-day intramuscular Jensen sarcoma or Walker 256 carcinoma was enhanced. The source of this enhanced glycoprotein synthesis did not, however, appear to be the liver, since no apparent increase in rate of glucosamine incorporation occurred in the microsomal fraction from tumor-host liver. Endotoxin, in

TABLE II. Percent of Total TCA-Soluble-C^{14} Found Nondialyzable.*

	Control			Jensen sarcoma			Endotoxin		
	30 min	60 min	120 min	30 min	60 min	120 min	30 min	60 min	120 min
Liver	.97	1.37	2.87	.98	1.51	2.08	1.39	2.25	5.51
Plasma	.20	.63	1.53	.23	.61	1.43	.60	—	61.6
Tumor	—	—	—	1.37	2.61	4.98	—	—	—

* Each value represents the average of 3 experiments; values express nondialyzable C^{14} as percent of total C^{14} found in the TCA-soluble fraction.

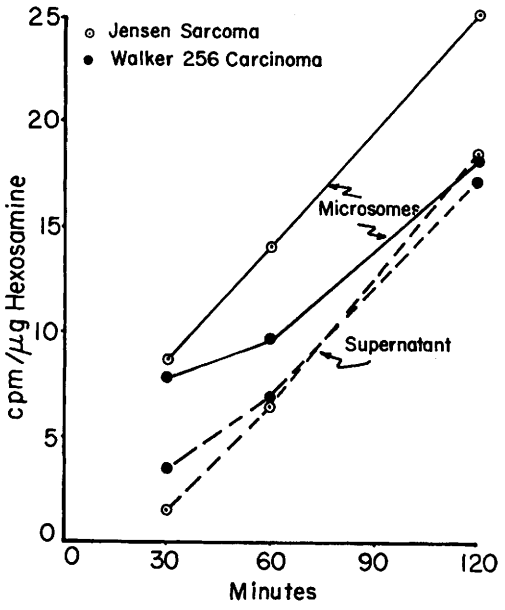

FIG. 3. Specific activity incoporated into tumor proteins. Solid lines are microsomal; broken lines supernatant. Jensen sarcoma, — ⊙ —; Walker 256 carcinoma, — . —.

contrast, elevated serum glycoprotein concentration, which was correlated with an increased hepatic incorporation of glucosamine. The rate studies might have been complicated by the presence of the increased endogenous pool of hexosamine in the tumor-host liver. This would appear, however, not to influence the rates of incorporation since the normal liver, which contained the lower hexosamine pool, had the higher specific activity, while the tumor host liver, with its increased pool size, had the lower specific activity. The influence of the pool size on the endotoxin-treated animals appears to have been overcome by its increased rate of glucosamine-C^{14} incorporation. While this work was in progress, Bekesi *et al*(6) reported that in Walker 256 carcinoma hosts the rate of incorporation of glucosamine-C^{14} into hepatic protein was not altered from normal in the early stages (6 days) of tumor development, but was enhanced at 12 days. They, as well as Molnar *et al*(7), working with the Ehrlich ascites tumor, presented evidence that the tumor synthesized glycoproteins which were subsequently released into host serum. A similar suggestion has been made by Spiers and Malone for hexosamines in patients with cancer(8). In the present study glucosamine-C^{14} was incorporated into the protein synthesizing apparatus of tumor.

The dialysis experiments showed that equivalent fractions of the control and tumor-host tissue were precipitated with TCA. Of interest, however, was that up to 62% of the total radioactivity of the TCA-soluble fraction from plasma of the endotoxin-treated animals was nondialyzable. This component was being synthesized, as an increase occurred with time, but the increase was greater in the endotoxin-treated animal.

The data presented here confirm our previous observation that the serum glycoprotein concentrations of animals hosting bacteria-free Jensen sarcoma and Walker 256 carcinoma were not elevated. Whether this is due to dilution *via* the increased plasma volume, an increased turnover of the glycoprotein in the tumor host, similar to that reported by Bekesi *et al*(6), or a combination of the two must await further study. The data presented would appear to support the hypothesis that enhanced hepatic biosynthesis of glycoprotein does not occur in the early stages of tumor growth (7 days).

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