milarity in tibial and hematological responses to both crude hypophyseal extracts and purified preparations would implicate the growth hormone molecule in effecting these alterations. However, since assay of other hypophyseal hormones contained in these crude extracts was not carried out their possible influence on the parameters chosen for study cannot be assessed here.

The failure to observe a linear response in body weight gain may be due in part to the short fasting time employed (and therefore variable intestinal contents), or may indicate a sensitivity of the hypophysectomized rat to the crude hetero-specie material injected.

Summary. Chicken and rat hypophyseal extracts were compared with a purified beef growth hormone preparation in the hypophysectomized, immature female rat using the tibial cartilage assay and hematological alterations. The avian adenohypophysis contains 1/8th of the growth-promoting material found in an equivalent amount of rat hypophyseal extract. Significant depression in hematocrit and WBSG levels occurs at doses of avian extracts which are unable to promote epiphyseal cartilage growth.

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Extraction of a Glucose Uptake Stimulator from Tumors.* (26830)

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The presence of a glucose uptake stimulator in a retroperitoneal fibrosarcoma has recently been reported from this laboratory(1). This tumor had been removed from a patient exhibiting repeated episodes of spontaneous hypoglycemia associated with increased plasma insulin-like activity. Following removal of the tumor, the patient became euglycemic and had plasma insulin-like activity indistinguishable from normal, which indicated that the tumor was the source of a glucose uptake stimulator(2). These observations suggested the possibility that a glucose uptake stimulator might be found in other neoplastic tissues even in the absence of hypoglycemic symptoms in the tumor-bearing patients. This paper reports the results of assays for a glucose uptake stimulator in various malignant tumors.

Methods. Tumors obtained from the operating room within minutes of removal from the patients were carefully dissected away from surrounding tissues, cut into pieces 3-4 cm thick, and stored at -20°C until use. In most cases it was possible to obtain tissues showing no histological evidence of neoplastic changes from the areas surrounding the tumor. Fractions were prepared from these tissues in the same manner as that used for

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the tumors and will be referred to as "normal" tissue fractions.

The fractions were prepared as follows: All manipulations were carried out at 3-5°C. Five g of tissue were homogenized in Krebs Ringer Bicarbonate buffer containing 2.5 mg/ml glucose(3). The homogenate was centrifuged and the supernatant brought to a 22% ethanol concentration. The precipitate was collected by centrifugation and was saved for assay. The supernatant was brought to a final ethanol concentration of 50% and the resultant precipitate was collected by centrifugation and saved for assay. For assay the precipitates were all suspended in buffer containing 2.5 mg/ml glucose by use of a Potter-Elvehjem homogenizer.

Assessment of the glucose-uptake stimulating activity (hereafter referred to as the stimulator) was made on the basis of an increase in glucose uptake by epididymal fat pads in the presence of a particular fraction over that in buffer alone. A control group was included in every incubation. No attempt has been made to quantitate the amount of stimulator in terms of units of insulin activity for the reasons given previously(1). Assays were carried out by measuring glucose uptake of intact fat pads from rats weighing 150-200 g. Glucose concentration was measured by the method of Somogyi(4). Details of the procedure have been reported(1).

Results. The data shown in Table I are representative assays of malignant tissues removed from different patients. Included are 3 different adenocarcinomas of the breast, 3 adenocarcinomas of the colon, one adenocarcinoma of the kidney, and one liposarcoma. "Normal" tissues including skin, colon, and kidney dissected from around the tumors were fractionated and assayed in a manner identical to that used on the tumors.

All 3 of the adenocarcinomas of the breast (Groups 1-3) yielded precipitates at both the 22%† and the 50% ethanol concentration which caused a significant increase in glucose uptake. The same was true of the adenocarcinoma of the kidney (Group 4) and 2 of the 3 adenocarcinomas of the colon (Groups 6 and 7). One colon tumor was without significant activity in either the 22% or 50% precipitate (Group 8). Most of the stimulating activity in the liposarcoma was concentrated in the 22% precipitate since addition of ethanol from 22 to 50% gave a precipitate with no significant activity.

Of the normal tissues assayed, neither the 22% nor the 50% precipitates of pectoral muscle nor skin stimulated glucose uptake significantly (Groups 9, 10, 13 and 14). In the case of the colon one specimen did not show a stimulating activity (Group 12) while another specimen showed stimulation in the 22% ethanol fraction (Group 11). Comparison of the activities in the normal colon tissue (Group 11) with the tumor tissue (Group 6), from this patient would indicate that the tumor had appreciably more stimulator than the normal tissue in both the 22% and the 50% ethanol precipitates. Kidney (Group 15) had as much activity as the kidney adenocarcinoma (Group 4) in the 22% fraction; however, increasing the ethanol concentration to 50% did not result in an increase in activity in the normal in contrast to the tumor.

Discussion. It is clear that all but one of the tumor fractions stimulated glucose uptake of the epididymal fat pad significantly. Corresponding fractions of normal tissue either had no stimulator or much less stimulator than did the tumors. It is immediately apparent that the stimulator has not been concentrated in a single fraction by the technics employed here. Previous experience with a retroperitoneal fibrosarcoma would have suggested that the stimulator could be concentrated by forming a 22-50% ethanol precipitate or a zinc acetate precipitate. This observation did not prove to be true except in isolated instances (Groups 2, 4 and 5). Also in contrast to our experience with the fibrosarcoma, it was not possible to remove a glucose uptake inhibitor from the fractions by dialysis. Continuing attempts to concentrate the stimulator by other procedures have not been markedly successful.

Most recent efforts to demonstrate a glu-

† The terms 22% and 50% ethanol precipitates refer to precipitates formed by addition of ethanol from 0-22% and 22% - 50%.

TABLE I. Glucose Uptake of Rat Epididymal Fat Pads Incubated in Ethanol Precipitates of Tumors and "Normal" Tissues, mg/g Fat Pad/3 Hours.

Group	Tissue	Control	22% ppt.	p*	Change‡	50% ppt.	p*	Change‡
			Neoplastic tissues					
1.	Breast Ca (P)	2.66 ± .28 (6)†	4.56 ± .47 (6)†	<.01	+1.90	5.70 ± .91 (6)†	<.02	+3.04
2.	" " (W)	2.42 ± .26 (5)	3.94 ± .42 (5)	<.02	+1.52	4.61 ± .17 (5)	<.001	+2.19
3.	" " (F)	.98 ± .08 (5)	2.81 ± .24 (5)	<.001	+1.83	2.05 ± .42 (5)	<.05	+1.07
4.	Kidney Ca (I.W.)	2.06 ± .17 (5)	4.19 ± .38 (5)	<.001	+2.13	6.33 ± .26 (5)	<.001	+4.27
5.	Liposarcoma (S)	2.65 ± .22 (5)	6.03 ± .81 (5)	<.01	+3.38	3.55 ± .63 (5)	n.s.	+ .90
6.	Colon Ca (Ba)	2.15 ± .33 (5)	5.11 ± .50 (5)	<.01	+2.96	4.10 ± .68 (5)	<.05	+1.95
7.	" " (Bu)	1.93 ± .26 (5)	3.43 ± .52 (5)	<.05	+1.50	3.65 ± .57 (5)	<.01	+1.72
8.	" " (C)	1.31 ± .31 (5)	2.25 ± .36 (5)	n.s.	+ .94	1.48 ± .04 (5)	n.s.	+ .17
			"Normal" tissues					
9.	Pectoral mus. (W)	2.42 ± .26 (5)	2.88 ± .61 (5)	n.s.	+ .46	3.02 ± .24 (5)	n.s.	+ .60
10.	" " (H)	1.01 ± .17 (5)	1.70 ± .28 (5)	"	+ .69	1.46 ± .17 (5)	"	+ .45
11.	Normal colon (Ba)	2.15 ± .33 (5)	3.98 ± .46 (5)	<.02	+1.83	3.15 ± .31 (5)	"	+1.00
12.	" " (C)	2.36 ± .31 (5)	2.14 ± .53 (5)	n.s.	− .22	2.58 ± .65 (5)	"	+ .22
13.	Skin (H)	1.89 ± .10 (5)	2.40 ± .85 (5)	"	+ .51	2.00 ± .50 (5)	"	+ .11
14.	" (W)	2.05 ± .37 (5)	2.69 ± .56 (5)	"	+ .64	2.42 ± .38 (5)	"	+ .37
15.	Kidney (I.W.)	2.06 ± .17 (5)	4.23 ± .55 (5)	<.01	+2.17	3.45 ± .41 (5)	<.02	+1.39

* p compares significance of the difference of means of experimental group to buffer control group. p is derived from t where

$$t = \frac{m_1 - m_2}{\sqrt{\left(\frac{\Sigma d_1^2 + \Sigma d_2^2}{n_1 + n_2 - 2}\right)(1/n_1 + 1/n_2)}}$$

n.s. = not significant

† Mean ± stand. error of mean. No. in parentheses is No. of fat pads in each group.

‡ Change is the difference of experimental group from control group.

cose uptake stimulator in tumor tissue have been directed toward a study of those tumors associated with spontaneuos hypoglycemia. Of the several attempts (see 5 for review) only 2 have been successful(2,6). Shortly after the discovery of insulin, however, several investigators demonstrated a material in tumor extracts not associated with hypoglycemia capable of modifying the carbohydrate metabolism of normal tissues. Waterman (7) showed that extracts of human carcinoma, mouse carcinoma, and sarcoma increased glycolysis of normal kidney slices and postulated the material acted at the first steps involved in glucose utilization. The tumor extracts had more of the glycolysis activator than did normal tissues. Brooks and Jowett(8) using a somewhat different procedure, failed to confirm this observation. Others demonstrated insulin-like activity in various malignant tissues(9,10) as well as in normal tissues(11). Most recently, Silverstein *et al.*(12) have demonstrated a hypoglycemic factor in leukemic tumors extractable with acetone but not with the usual insulin extraction procedure.

The data reported here and the observations referred to above suggest that a material capable of stimulating glucose uptake *in vitro* is associated with at least several different malignant tissues and perhaps some normal tissues. With the extraction method employed here there is more stimulator present in tumors than in surrounding, presumably, non-neoplastic tissue.

A discussion of the identity of the glucose uptake stimulator is not now profitable for two reasons. The stimulator has not been adequately concentrated and purified. Secondly, it has been demonstrated that glucose uptake of epididymal fat pads is stimulated not only by insulin(13), but also by growth hormone(14,15), ACTH(15,16), and epinephrine(16,17). Because of these observations, and in view of the differences in activity in comparable fractions of the different tumors reported here, it is entirely possible that more than one factor is responsible for stimulation of glucose uptake.

Summary. A material capable of stimulating glucose uptake of rat epididymal fat pads *in vitro* has been extracted and concentrated by ethanol precipitation from several neoplastic tissues. Most of the normal tissues assayed did not have significant amounts of the glucose uptake stimulator. In the 2 cases where a stimulator was found in normal tissues, it was present in lower concentrations than in the corresponding tumors.

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