Effect of Insulin on Free Tumor Cells in Peritoneal Exudate and on Their Host.* (22161)

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Soon after discovery by Warburg(1) of the high rate of glycolysis in tumors, several attempts were made to inhibit growth of tumors by influencing their sugar metabolism. Thus, Silberstein *et al.*(2) reported delay or complete inhibition of tumor growth in tarred animals after insulin treatment. This inhibition of carcinogenesis by insulin was observed also under various experimental conditions by von Witzleben(3), Muenzner and Rupp(4) and by Risse and Poos (quoted by Bauer(5)). Moreover, Braunstein(6) recorded for diabetics with tumors, better glucose tolerance and decrease of blood sugar level following increase in tumor size. However, Bauer(5) could not detect any variations of sugar metabolism in cancer patients or any change in their condition by variations of sugar level. This discrepancy in results was perhaps responsible for the lack of further publication on this problem as a careful search of the literature did not reveal any recent data on insulin effect in tumor-bearing animals. However, some statistical research has been done on the association of cancer with diabetes suggesting a possible relationship between sugar metabolism and malignant growth(7, 8,9).

The method of free tumor cell culture in peritoneal fluid(10-12) provided a useful tool for quantitative estimation of the effect of various factors on tumor cell growth. The use of globin insulin assured more continuous and uniform changes in blood sugar level. This selection of technic and material enabled us to obtain some quantitative information about insulin action on tumor cells and on their hosts.

Material and methods. Tumors Sarcoma-180 and Sarcoma-37 were grown by serial intraperitoneal transfers in CFW (Carworth Farms) mice. All normal mice treated with insulin were of the same strain and age. Doses of 10^6 to 10^7 tumor cells from peritoneal exudate were inoculated intraperitoneally into mice. In each experiment, 20 mice remained untreated and 20 received daily intramuscular injections of insulin doses (0.25 to 2 units). Peritoneal exudates were obtained for total and differential cell counts on 4th and 5th day of tumor cell growth by technic previously described(10). For 10 mice of each series the percentage of mitoses in tumor cells was recorded in addition to differential counts. Similar examinations were carried out on exudate induced in 20 control mice by injection of normal tissue (liver, spleen) suspensions. In other series, inoculated and normal mice were treated simultaneously with daily insulin injections for 5 days and their survival span was recorded.

Results. Results of total, differential, and calculated tumor cell counts in peritoneal exudates from untreated and insulin treated animals are recorded in Table I.

The data of Table I show that average concentration of tumor cells was not influenced significantly by 3 insulin doses of 0.5 unit (series IA). It was decreased markedly by doses of 1 or 2 units (series 1B, 1C, 2A, 3A, 4A, 5A, and 6A). This effect was due to decrease either of total exudate cell number (3A and 5A) or of the proportion of tumor cells (2A, 6A) or to both (1B, 1C, 4A). This effect could be prevented by injection of massive doses of glucose immediately after insulin: the same dose of glucose alone did not induce any variations in cellular formula of exudate. Thus both total number of cells and number of tumor cells are apparently stimulated in the presence of both insulin and glucose. Percentage of mitoses in tumor cells of controls was 9 to 19 (average 12) and in in-

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TABLE I. Growth of Free Tumor Cells in the Peritoneal Cavity of the Mouse under Insulin Treatment.

Exp. No.	Tumor inoculated, i.p.	Treatment with insulin: Days	Units daily	Results (averages and variation extremes): Amt of peritoneal exudate	No. of cells (tumor & leukocytes)/mm³	% tumor cells	Calculated No. tumor cells/mm³
1 A	S- 37	3	.5	3+	118	46	55
				2+–3+	53–191	32–60	27– 88
B		3	1	2+	92	39	34
				+–2+	60–112	14–53	15– 49
C		3	2	2+	46	36	15
				+–2+	32– 77	18–53	5– 23
D		0	0	3+	123	52	55
				2+–3+	72–157	41–78	43– 79
2 A	S-180	4	2	2+	23	21	5
				+–3+	19– 28	11–26	3– 7
B		0	0	3+	26	53	16
				2+–3+	16– 27	23–75	4– 45
3 A	"	4	1	2+	146	42	63
				+–3+	56–213	34–57	25–113
B		0	0	3+	193	39	78
				2+–3+	48–320	30–47	14–134
4 A	"	5	1	+	69	59	43
				+–2+	51– 91	30–72	15– 65
B		0	0	3+	109	68	77
				2+–3+	75–142	57–80	44–114
5 A	"	3	2	2+	117	28	31
				+–3+	70–173	11–55	8– 53
B		0	0	3+	280	31	76
				2+–3+	189–419	16–68	39–129
6 A	S- 37	4	1	2+	139	17	25
				+–3+	88–180	11–28	10– 39
B		4	1	3+	194	37	68
		1 cc 20% glucose daily		2+–3+	135–317	19–58	35–102
C		4	0	3+	84	38	32
		1 cc 20% glucose daily		2+–3+	36–188	25–62	10– 68
D		0	0	3+	105	31	35
				2+	39–156	10–50	11– 56

20 mice in each group. For total numbers and differential counts, averages and extremes are indicated. Amount of exudate estimated* as proportionate to volume of fluid obtained by insertion of glass capillary in peritoneal wall: 3+ = .5 mm³ or more, 2+ = <.5 but >.1 mm³, + = <.1 mm³, 0 = no fluid.

* Reliability of this estimate was shown by comparison with the amount of exudate measured at autopsy.

sulin treated—0 to 11 (average 5). No significant variations (more than 10% of the average) in the leucocyte formula were noted in the exudate induced by normal tissue suspension.

Effect of insulin on host of tumor growth was estimated by comparing the life span in 3 groups of mice: 1) tumor-bearing and untreated, 2) tumor-bearing and insulin treated and 3) insulin treated normal mice. The results are reported in Table II.

It appears from Table II that mice of group 2 died earlier than in control groups 1 and 3. Administration of glucose after insulin decreased mortality rate in group 3, but not in group 2. Doses of 0.25 unit of insulin were insufficient to influence survival span of mice.

Analogous experiments were carried out in mice bearing subcutaneous tumors with similar findings but with a wider range of variations.

Discussion. Decrease in number of free tumor cells in the exudate after insulin treat-

TABLE II. Survival of New and Tumor-Bearing Mice Treated with High Doses of Globin Insulin.

	Mice with tumor growth					Mice without tumor growth (new mice)			
	Treated daily with requisite doses (units) of insulin								
	Group 1	Group 2				Group 3			
Days after 1st insulin inj.	Untreated controls (tumor growth, no insulin)	Tumor growth and insulin treatment				Insulin treatment, no tumor growth			
		.25	.5	1-2	1 + glucose	.25	.5	1	1 + glucose
3	20	20	18	15	20	20	17	15	18
5	19	18	12	13	12	20	15	13	16
7	14	16	7	8	7	20	12	11	14
10	11	9	2	1	2	20	12	11	14

Mice were inoculated intraperitoneally with 10^6 S-180 cells 4 hr before 1st insulin injection. Insulin given 5 successive days. 20 animals in each series. Dose of glucose: 1 cc of 20% solution. Number of surviving mice indicated.

ment could neither be interpreted as effect of dilution, since volume of exudate was reduced (Table I), nor as increase in proportion of leucocytes, because insulin did not modify the cellular formula of exudate in controls. Moreover, decrease in percentage of mitoses indicated the effect of some factor in the peritoneal fluid on the floating tumor cells. This factor was either insulin or some metabolic product of hypoglycemia, since glucose prevented cell damage.

Growth inhibition of tumor cells by insulin excluded obviously the possibility of incriminating malignant growth for early death of their hosts; on the other hand, comparison with results in insulin treated normal mice pointed out that sensitivity to insulin was increased by occurrence of malignant growth. Since the lethal effect of high insulin doses was partly reversible by glucose in normal mice and irreversible in tumor hosts, it may be presumed that in the latter some vitally important tissues were damaged by hypoglycemia and its products, or by insulin itself; for instance, it may be presumed that insulin increased cell permeability(13,14) for some toxic metabolites of malignant growth. Since combined treatment with insulin and glucose induced far greater effect on exudate and tumor cells than simple reversal of insulin action, a further study of this phenomenon may offer new insight in our problem.

Summary and conclusions. CFW mice were inoculated i.p. with S-180 or S-37 cell suspensions and treated with 3 daily i.m. injections of globin insulin (0.5 to 2 units). On the 4th or 5th day, specimens of peritoneal exudate were withdrawn from these mice and from untreated controls; the number of tumor cells per cmm and the percentage of their mitoses were consistently lower in insulin treated animals; this effect of insulin could be prevented by repeated injections of glucose (1 cc of 20% solution). In other experiments, the survival span was recorded for insulin treated tumor-bearing animals, their untreated controls and insulin treated normal mice. The first group showed the shortest survival which could not be extended by injections of glucose. It is concluded that growth of free tumor cells in the exudate was reversibly inhibited by hypoglycemia, its metabolic products or by the direct effect of insulin on tumor cells (permeability?) and that the same factors (or some of them) provided additional stress to some vitally important tissues damaged by tumor growth.

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Antifibromatogenic Activity of 19-Nor-α-ethinyltestosterone in the Guinea Pig.* (22162)

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Both the progestational and antifibromatogenic potencies of 19-nor-progesterone (19-nor-P) are greatly superior to those of progesterone (P)(1,2). Likewise, the progestational potency of (19-nor-α-ethinyltestosterone (19-nor-ET)(3) has been found to be 5 times that of α-ethinyltestosterone (ET) (4). ET also is antifibromatogenic though about 15 times less than P(5,6). Because of the clinical importance of 19-nor-ET which is active by mouth(4) its antifibromatogenic activity was studied.

Methods previously described(2) were used. Because of slow absorption of ET (0.2 to 0.4 μg/sq mm of the pellet) 4 to 8 pellets were implanted subcutaneously. Absorption rate of 19-nor-ET was 1.6 μg/sq mm. Absorption rate of 19-nor-P also was greater than that of P (7 against 3 μg/sq mm(2,5,7).

Results with ET and 19-nor-ET are summarized in Table I. No prevention of estrogen-induced abdominal fibroids was obtained with the quantities of ET used. On the contrary, with the same quantities of 19-nor-ET the fibrous reaction was greatly diminished; the same occurred even with as little as 50 to 70 μg/day (see individual values in parentheses). With ET values of 4 to 7 may still occur with 150 to 210 μg/day(5). Thus the antifibromatogenic potency of 19-nor-ET is probably more than 4 times that of ET. It is noteworthy that the increase from ET to 19-nor-ET is of the same quantitative order as from P to 19-nor-P(2). However, 19-nor-ET is far less potent than 19-nor-P and even than P.

There was also a very pronounced increase in prevention of abnormal uterine weights

TABLE I. Antifibromatogenic and Antihysterotrophic Activities of ET and 19-nor-ET in Castrated Female Guinea-Pigs with Subcutaneously Implanted Pellets of Estradiol (Absorption: 47 to 50 μg/day). Duration of experiment: 3 mo. For classification of results see (6,8).

Steroid tested		No. of	Fibrous		
Compound	μg/day	animals	tumoral effect*	Q_{2-3}†	Uterine wt, g
ET	71 (35–127)‡	10	6 ± .9‖	1.9	6.0 (2.7–8.7)
19-nor-ET	80 (49–126)§	14	2 ± .2¶	.3	2.6 (1.5–4.0)

* Arbitrary values for number and size of tumors, ranging from zero to 12(6).
† " " " *large* tumors only, per animal (8).
‡ 4 to 8 pellets.
§ ½ to 1 pellet.
‖ Individual values: 2–2.5–3–4–5.5–6.5–7.5–7.5–8.5–11.
¶ " " : (1.5–1.5)–1.5–1.5–1.5–1.5–1.5–(2–2)–2–2.5–(3–3)–3. In parentheses: animals receiving only 50-70 μg/day of 19-nor-ET.

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