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Glucose, Mannose, and Fructose Metabolism by Ascites Tumor Cells: Effects of Dinitrocresol. (21185)

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The present experiments are part of a program dealing with the metabolism of ascites tumor cells derived from the Ehrlich carcinoma(1) or from sarcoma 180. Such tumor cells are now under wide investigation because of the potential advantages which they offer over tumor slices or homogenates(2-6). The relative rates of utilization of glucose, mannose, and fructose have been measured, with and without addition of different concentrations of 4,6-dinitro-o-cresol (DNC).

Methods. Ehrlich carcinoma. The ascites tumor was maintained in male or female mice of the Cox strain. On the 7th to the 9th day after inoculation, each mouse was anesthetized with ether or chloroform and the ascites fluid aspirated, without anticoagulant, into a pre-chilled receptacle; the fluid from 12 mice was pooled. The crude ascites fluid was diluted with 4 volumes of a cold solution (Solution A) of the following composition: NaCl, 0.14 M; KCl, 0.006 M; $CaCl_2$, 0.003 M; $MgCl_2$, 0.001 M; glycylglycine, 0.025 M; pH adjusted to 7.4. The diluted cell suspension was filtered through 2 layers of cheesecloth and centrifuged lightly in tared centrifuge tubes (Sorvall medium centrifuge, Model SP/X) by bringing the speed to 1300 R.P.M. over a period of 3 minutes and then shutting off the power. The supernatant fluid was drawn off and discarded, removing most of the red and white blood cells and some unsedimented ascites cells. The sedimented cells were resuspended in the same solution, using a volume 5 times that of the original ascites fluid. The suspension was centrifuged as before and the supernatant fluid discarded. The sedimented ascites cells were resuspended in Solution A, making the total volume twice that of the original ascites fluid; the ascites cells were then collected in a packed layer by bringing the speed to 2500 R.P.M. over a period of 5 minutes and shutting off the power. The weight of the packed cells was ascertained by weighing in the tared centrifuge tubes. The weighed cells were finally suspended in Solution A to give a concentration of 250 mg wet cells per cc of total suspension.

Anaerobic consumption of glucose, mannose, and fructose by the ascites tumor cells was substantially larger in isotonic NaCl or a balanced salt solution than in isotonic KCl.

The experiments were carried out in Warburg flasks: main compartment, 0.3 cc glycylglycine buffer (0.25 M), 0.1 cc $KHCO_3$ (0.02 M), 1 cc hexose solution to give the concentration desired,* 0.6 cc solution of 4,6-dinitro-o-cresol (DNC) to give the final concentrations shown; side arm: 1 cc cell suspension; center cup, 0.3 cc 5 N NaOH

* Hexose concentrations were 5-10 millimolar. Rates of utilization were not increased by using higher substrate concentrations.

TABLE I. Glucose and Fructose Used and Lactate Formed by 250 mg Wet Weight Washed Ascites Tumor Cells (Ehrlich Carcinoma and Sarcoma 180). Results are expressed in micromoles. Temp. 25°C.

Incubation time, min.	Molar conc. DNC $\times 10^{-5}$	Ehrlich ascites carcinoma				Ascites sarcoma 180			
		Glucose substrate		Fructose substrate		Glucose substrate		Fructose substrate	
		Glucose uptake	Lactate formed	Fructose uptake	Lactate formed	Glucose uptake	Lactate formed	Fructose uptake	Lactate formed
		Under aerobic conditions							
10	0	2.7	8.2	2.3	8.0	0	6.6	.3	5.1
"	1.6	7.7	14.9	3.3	7.5	5.7	10.6	−1.0	5.0
"	3.2	10.5	16.4	2.3	7.7	9.0	15.0	− .7	5.0
20	0	4.3	11.7	5.0	9.8	4.3	8.6	2.3	5.8
"	1.6	9.3	21.7	4.5	11.2	11.0	16.3	1.7	6.8
"	3.2	11.3	25.4	5.0	9.8	18.7	22.2	5.3	6.8
40	0	5.3	15.8	5.0	13.5	4.7	10.9	4.0	7.6
"	1.6	15.1	31.0	5.7	15.3	14.7	24.8	5.3	9.9
"	3.2	21.3	31.9	8.7	16.7	22.6	28.3	6.7	10.4
		Under anaerobic conditions							
10	0	5.5	11.2	1.0	7.5	3.0	9.1	.9	7.1
"	1.6	6.0	13.0	1.5	6.9				
"	3.2	8.3	14.6	2.8	7.4	3.7	11.1	1.3	6.0
20	0	8.7	16.5	2.2	11.2	6.2	14.4	3.5	10.2
"	1.6	11.7	23.0	3.8	10.3				
"	3.2	12.0	24.9	3.0	11.1	7.7	18.9	2.7	10.5
40	0	12.7	25.8	6.5	18.7	9.4	23.9	7.5	16.2
"	1.6	18.3	32.2	6.2	18.0				
"	3.2	19.7	33.5	6.8	18.2	12.0	29.9	6.7	15.5

Initial conc., μM:	Carcinoma	Sarcoma		Carcinoma	Sarcoma
glucose aerobic	27.7	28.7	fructose aerobic	30.3	29.0
" anaerobic	33.7	29.3	" anaerobic	32.5	32.7

(for aerobic experiments only); gas phase, air or nitrogen. Up to this point all procedures were conducted at 5°C. The flasks were shaken at 25°C, gassed, closed, equilibrated for 10 minutes. The cells were tipped in from side arm and observations begun. After the desired incubation the flasks were transferred to an ice bath and the following aliquots removed: 1 cc for lactate analysis(7); 1 cc for hexose determination(8); the remainder for determination of final pH. Suitable zero time samples were prepared and handled similarly without incubation.

Sarcoma 180. The original inoculum of Crocker sarcoma 180 in the ascites form was obtained by courtesy of Dr. Kanematsu Sugiura, of the Sloan-Kettering Institute. The tumor was maintained in male or female Sutter Swiss mice, the ascites cells being obtained from each mouse on the 7th day after inoculation. These ascites cells were obtained and handled throughout by exactly the same procedures as were used for the Ehrlich ascites cells.

Results. Hexose consumption and lactate formation for 3 time periods, with glucose or fructose as substrate, are shown in Table I. The total uptake of glucose, particularly for the longer periods of time and under the stimulating influence of dinitrocresol, was much higher than that of fructose. It should also be noted that under both aerobic and anaerobic conditions the uptake of glucose and the lactate production therefrom were markedly increased by addition of dinitrocresol, while relatively little effect was exerted by the same agent on the uptake of fructose and the production of lactic acid therefrom. The rates of hexose consumption and lactate formation with glucose, mannose, or fructose as substrate, without and with the addition of 7 concentrations of dinitrocresol, are shown for the Ehrlich ascites carcinoma cells in Fig. 1 and for the ascites sarcoma cells in Fig. 2. The values for glucose were obtained with a

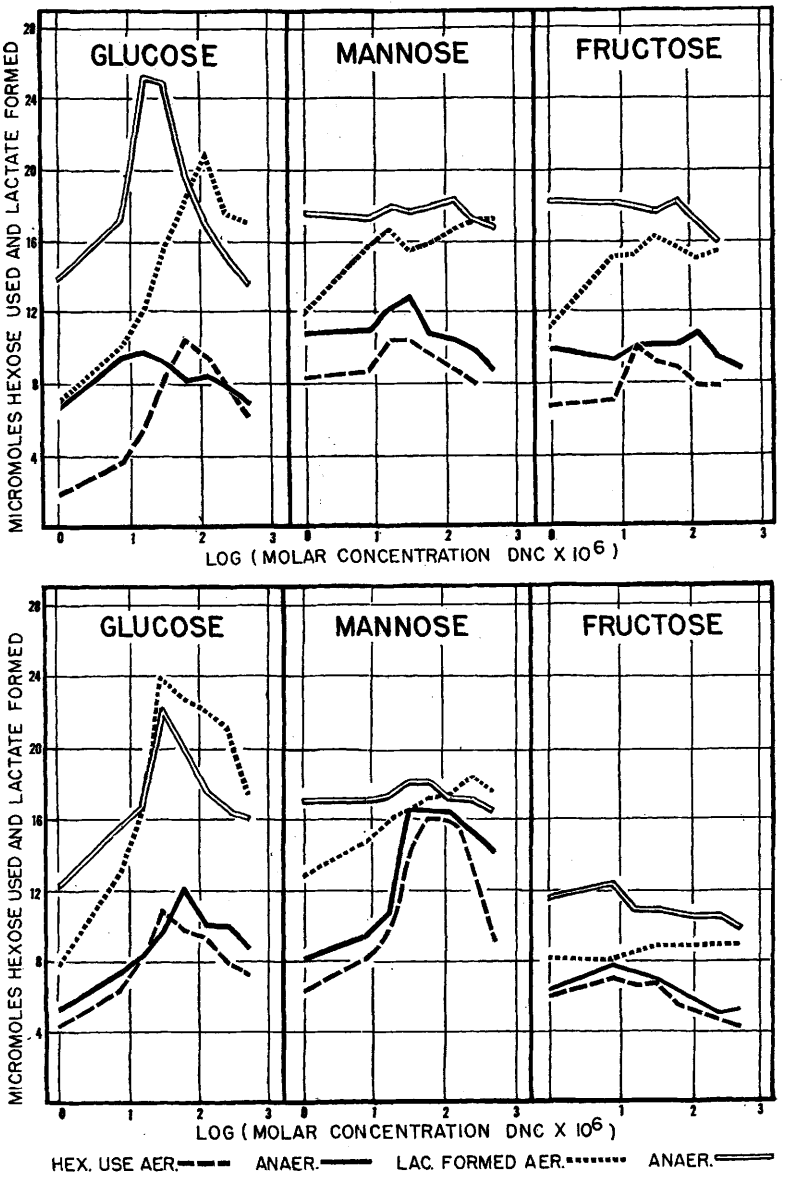

FIG. 1 (top). Hexose uptake and lactate production by Ehrlich ascites carcinoma cells under aerobic and anaerobic conditions and effect exerted by different concentrations of 4,6-dinitro-o-cresol (DNC). Results for glucose were obtained with a 10-min. period of incubation, those for mannose and fructose with a 40-min. period, these being the maximum time periods which could be employed without exhaustion of hexose. Initial hexose concentrations were: glucose, 13.2 μM; mannose, 28.9 μM; fructose, 30.4 μM. Incubation at 25°C.

FIG. 2 (bottom). Hexose uptake and lactate production by ascites sarcoma 180 cells. Experiments were run and recorded as described for Fig. 1. Initial hexose concentrations were: glucose, 14.3 μM; mannose, 26.0 μM; fructose, 30.4 μM.

10-minute period of incubation; those for mannose and fructose with a 40-minute period of incubation, preliminary experiments having been made to establish these as the maximum time periods which could be employed without decrease in rate due to exhaustion of hexose. Utilization of glucose per hour anaerobically for both the carcinoma and sarcoma cells was over twice that for fructose.

Neither type of tumor cell produced any

lactate, either aerobically or anaerobically, with or without DNC, when used without addition of hexose. From further experiments it was found that both types of tumor cell were incapable of taking up galactose, arabinose,† or xylose, either aerobically or anaerobically, with or without the addition of DNC, and that no lactic acid was produced from any of these sugars.

With the carcinoma, and still more markedly with the sarcoma, the aerobic and anaerobic uptake of glucose was increased by addition of dinitrocresol, reached a peak, and subsequently fell; furthermore, the aerobic and anaerobic production of lactate rose markedly with increasing concentrations of DNC, reached a peak, and subsequently fell. With glucose as substrate, the number of micromoles of lactate produced was approximately twice the number of micromoles of glucose taken up. There was marked poisoning of the Pasteur effect, confirming and extending previous observations on tumor slices(9-11).

The uptake of mannose by both the carcinoma and sarcoma cells appeared, under both aerobic and anerobic conditions, to be increased by addition of DNC, with a subsequent fall at higher concentrations. However, precise values for mannose consumption cannot be given because the method of analysis is not completely satisfactory under the present experimental conditions. The lactate production from mannose aerobically was increased by addition of DNC and did not appear to suffer any diminution at higher concentrations of DNC. The lactate production anaerobically, which started at a high level, showed no appreciable increase under the influence of DNC.

The consumption of fructose was not significantly increased by DNC. The lactate production aerobically in the carcinoma series increased under the influence of DNC and did not fall at high concentrations. The anaerobic lactate production from fructose showed the same effect as was observed in the case of mannose, a fairly high production of lactate in the absence of DNC, which was not appreciably changed on addition of various concentrations of DNC. The oxygen consumption of the tumor cells was increased with increasing concentrations of DNC to a maximum and then decreased to about the original level, even in the absence of added substrate. The addition of glucose had no effect and that of mannose had only a slight effect upon the rate of oxygen consumption at any dinitrocresol concentration; addition of fructose permitted a somewhat higher oxygen consumption, particularly at the optimal concentration of DNC (Fig. 3). It will be noted that the fraction of hexose disappearance accounted for by oxygen consumption is very small, in agreement with the fact that the lactate production usually accounts for nearly all hexose disappearance.

Discussion. The present findings regarding hexose use by ascites cells are of particular interest in two respects. First, the relative rates of glucose, fructose, and mannose utilization differ markedly from those to be expected from current studies on hexokinases of other animal and plant cells; in the present instance the relative rates for glucose, fructose, and mannose are approximately 2:1:1, respectively, as compared to 1:1.4:0.5 for yeast or brain hexokinase(12,13), and 1:1.8:1.2 for Arbacia egg hexokinase(14). Further studies are required to establish whether the type and amount of hexokinase in ascites cells can account for these differences or whether the use of hexoses is determined by their relative rates of entrance into these cells.

The second finding to be stressed is the difference in the effect of DNC upon anaerobic utilization of glucose or mannose on the one hand, and of fructose on the other. The stimulation of anaerobic glucose consumption by DNC here observed is one of the largest obtained with any animal or plant cell (See Simon(15) for review); anaerobic use of mannose is also markedly stimulated by DNC, particularly in the sarcoma 180 ascites cells. In contrast, anaerobic use of fructose is *not* stimulated by DNC, although the basal rate for fructose is approximately the same as for

† Lactate production by the ascites carcinoma cells from 0.01 M glucose was reduced approximately 96, 80, and 50% when arabinose was concurrently present at concentrations of 0.3, 0.2, and 0.15 M, respectively. These results suggest a corresponding diminution of glucose uptake.

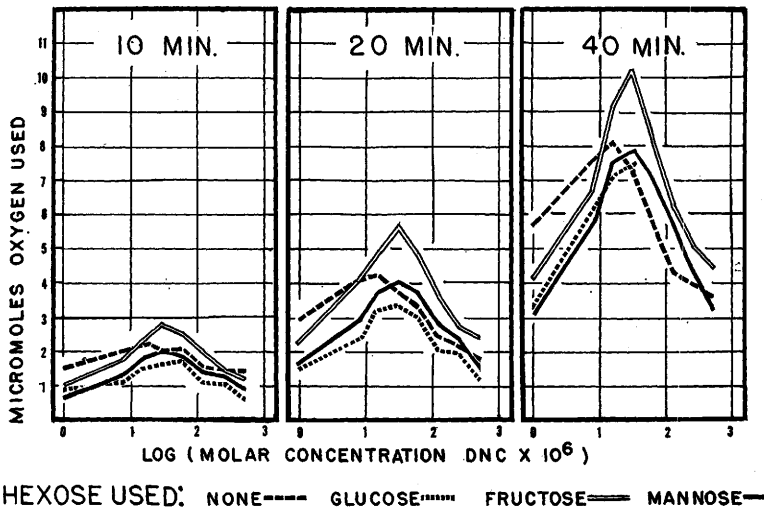

FIG. 3. Oxygen consumed by 250 mg wet wt washed Ehrlich ascites carcinoma cells without addition of hexose and with 10 millimolar glucose, fructose, or mannose. Incubation at 25°C.

mannose. Thus, it would appear that the pathway for utilization of fructose in these cells is not subject to the same regulatory mechanisms as those for glucose and mannose; further experiments to elucidate the nature of this difference are under way.

A third point of some interest is that, whereas the number of micromoles of lactate formed from glucose or fructose is generally approximately twice the number of micromoles of hexose used, lactate production accounts for only about half of the mannose used at optimal concentrations of DNC. This suggests that mannose isomerase may be a limiting factor for conversion of mannose to lactate in these ascites cells.

Summary. 1. Ascites tumor cells derived from Ehrlich carcinoma or sarcoma 180 consume glucose, mannose, or fructose, but not galactose, xylose, or arabinose. Under anaerobic conditions the hexose consumed is approximately accounted for by lactate produced. 2. The consumption of glucose and the production of lactate therefrom under anaerobic conditions are markedly stimulated by dinitrocresol. In contrast, the anaerobic consumption of fructose and of lactate production therefrom and the anaerobic production of lactate from mannose are not stimulated by DNC. 3. The aerobic consumption of glucose and production of lactate therefrom are raised by DNC to nearly the anaerobic levels; there is thus strong poisoning of the Pasteur effect. The aerobic consumptions of mannose and fructose are less influenced by DNC, but the production of lactate therefrom is raised at higher concentrations of DNC to the anaerobic level. 4. The oxygen consumption of both carcinoma and sarcoma cells, with and without addition of DNC, is virtually unaffected by the presence or absence of assimilable hexoses.

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Renal Hemodynamics During Erect Lordosis in Normal Man and Subjects with Orthostatic Proteinuria.* (21186)

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Orthostatic proteinuria has been attributed to a marked reduction in renal blood flow occurring characteristically in this condition during standing(1-4). The inference that there is a difference in hemodynamic postural response between orthostatic proteinuria patients and normal individuals is based on inadequate evidence chiefly utilizing urea and creatinine clearance methods.

Smith(5) described reduction in renal plasma flow and glomerular filtration rate in a normal subject in the passive erect posture (tilt). The confirmatory data of Brun, Knudsen and Raaschou(6) are of equivocal significance since observations were made during periods of marked change in urinary volume, and some specimens were collected without urethral catheterization. In the present study observations on renal hemodynamics in recumbency and erect lordosis have been made in normal subjects and individuals with orthostatic proteinuria under conditions providing relatively constant rates of urine flow. The findings suggest that there is no significant difference in the renal hemodynamic response of these two groups.

Methods. Glomerular filtration rate (GFR) as measured by inulin clearance and renal plasma flow (RPF) as measured by p-aminohippurate clearance were determined in 5 normal young adult males and 5 with orthostatic proteinuria. Observations were made with subjects in a basal state and under moderately hydropenic conditions. Clearances were determined during three 20-minute control periods with the subject in the supine position and during 15 to 20 minutes in erect lordosis. All urine specimens were obtained by urethral catheterization and analyzed for protein as well as for the clearance substances. Inulin was determined by a modification of Harrison's method(7); p-aminohippurate by the method of Smith and associates(8). Quantitative urinary protein was determined by the biuret method of Foster, Rick and Wolfson(9) in those specimens exhibiting protein with 20% sulfosalicylic acid [>10 mg %(10)].

Results. In the 5 normal individuals (Table I) GFR during lordosis decreased an average of 29%, ranging from −6% to −56%; RPF an average of 37% with a range from −9% to −55%. The filtration fraction was unchanged in 2 and increased in 3. Urinary volume (V) decreased an average of 41% with a range from −7 to −67%. Urinary protein did not appear during or after erect lordosis.

In the 5 subjects with orthostatic proteinuria (Table II) GFR during lordosis decreased an average of 17% with a range of +6% to −40%; RPF an average of 27%, ranging from −11% to −42%. The filtration fraction was unchanged in 2 and increased in

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