Influence of Glucose Antimetabolites on the Walker Tumor*

HOWARD A. BALL, ARNE N. WICK, AND CURTIS SANDERS

(Biological Research Institute, San Diego Zoological Society, San Diego, and Scripps Clinic and Research Foundation, La Jolla, Calif.)

A large number of antimetabolites of nucleic acid components have been tried as antitumor agents (11). While some have retarded tumor growth, they are in general toxic to the host animal. This is perhaps to be expected, since protein synthesis is an essential phenomenon in all tissues.

In the studies to be described, we have attempted to interfere with the energy sources of the cell. Since the utilization of glucose by the glycolytic pathway appears to be more important for the malignant than the normal cell, the effects of glucose antimetabolites on tumor growth were studied at dosage levels which were not obviously toxic to the animal.

Glucosamine has been tried as an antitumor agent by a number of workers, with inconclusive or negative results (2, 4, 6–8, 12, 13, 15–17) after the original promising observations of Quastel and Cantero (10). In most cases, however, a single daily dose of the test material was used. 2-Deoxy-D-glucose (2DG) appears to have produced some suppression of tumor growth or glycolysis (5, 14, 20, 21). Since compounds of this type are readily excreted by the kidney, effective levels of the antimetabolite were probably present for only a very short time. In the present studies, we have used multiple daily doses of glucosamine and 2-deoxy-D-glucose to achieve a more constant exposure to the compounds. The amount of glucosamine employed was generally at least double that reported by other observers. Both compounds produced significant reductions in tumor growth at levels which did not seriously affect the health of the host.

EXPERIMENTAL

Glucosamine studies.—Glucosamine hydrochloride from two sources was used either as received or, in some instances, after recrystallization. Fragments of Walker tumor 256 were transplanted by trocar into the left axillary region of young (120–160-gm.) Wistar rats. Intraperitoneal injections of glucosamine hydrochloride (5–25 per cent) were made from 4 to 6 times/24 hr (greatest interval was 8 hr.) A few controls were untreated, but most received glucose or saline in concentrations comparable with those given the treated group and on the same divided dose schedule. Animals were sacrificed and tumors weighed on the 19th day after inoculation.

Table 1 presents a summary of various experiments with 400–600 mg. glucosamine hydrochloride/rat/day. This approximates the range of 2,000–3,000 mg/kg/day, or about twice the amounts used in screening studies. A consistent but mild reduction of tumor weight is apparent. Many times, with the small populations involved, these differences do not meet the requirements that assure statistical validity. If, however, these groups are combined on the basis of assigning a 100 per cent value for mean tumor weight of any control group and determining the value of treated tumors in terms of per cent of controls, we find that, for a combined group of 70 controls of all types compared with 65 glucosamine-treated animals processed concurrently with the controls, the mean weight of tumors in the treated group is 73.3 per cent that of the controls. This difference is 5.27 times the standard error of the difference (Table 2).

All treated animals gained in net body weight during tumor growth but not to the same degree exhibited by the controls. A correlation study between increment of body weight and tumor weight in a series of 238 observations on single implants of the Walker tumor previously recorded (correlation coefficient = .01) may be interpreted to indicate that the difference in carcass weight gain between treated and control groups reported here cannot account for the changes in tumor size observed in the treated group.

Divided doses of less than 400 mg/rat/day may at times produce a statistically valid effect but in general cannot be consistently duplicated. A com-

* Supported in part by a grant from the California Division, American Cancer Society.

Received for publication November 20, 1956.

bined group of animals receiving 200–400 mg/rat/day (about 1,000–2,000 mg/kg/day) is presented in Table 2. In view of the findings with higher doses, and interpreted in the light of that evidence, it seems probable that this lesser effect is also due to administered glucosamine.

2-Deoxy-D-glucose studies.—2DG was prepared according to the method of Cramer (3) and recrystallized (m p., 147°–149° C., specific rotation $[a]^{20°}$ D + 46.3° [C = 1.0], 3-hr. equilibrium; Van Slyke reduction, 36 mg. glucose equivalent at 8′, 136 mg. at 16′.)

corresponding paired animals received an equivalent amount of glucose on the same injection schedule. At intervals during the growth period, measurements of tumor were made, and the mean tumor diameter in mm. was calculated and plotted. Mean tumor weights are expressed with standard deviations.

In the residue of animals from the pairing of the major groups, a group with small tumors on day 10 were found useful as untreated controls for further comparison with the small group treated with 2DG during the first 10 days.

TABLE 1

EFFECT OF HIGH DIVIDED DAILY DOSAGE OF GLUCOSAMINE ON WALKER TUMOR

Individual Experiments

		Controls				Glucosamine HCl				
Total dose (mg/rat/day)	Times/day	No. rats	Solution and concentration (per cent)	Mean tumor wt.* (gm.)	Mean increment body wt/rat (gm.)	No. rats	Glucosamine concentration (per cent)	Mean tumor wt.* (gm.)	Mean increment body wt/rat (gm.)	Diff. / S.E. diff.
440	4	12	G† 21	16.0±1.7	11	14	21	11.1±1.2	6	2.4
400 Insulin 2	4	15	G 42	15.5±1.4	93	15	25	10.1±0.6	60	4.5
450	6	7 14	G 21 S† 6.8	15.5±1.3	63	14	21	11.9±1.0	56	2.1
600	4	11	G 5	22.6±2.1	72	14	5	18.0±1.9	61	1.6
600	4	11	G 5	13.4±1.7	85	8	5	10.4±0.9	76	1.5

* Mean tumor weights on 19th day of growth are expressed with standard errors. All animals were treated from day following inoculation to day before sacrifice.

† G: Glucose; S: Saline.

TABLE 2

COMPARISION OF ANTITUMOR EFFECT OF HIGH AND LOW DIVIDED DAILY DOSAGE OF GLUCOSAMINE ON WALKER TUMOR

Combined Groups

	Controls		Glucosamine HCl		
Total dose Glucosamine HCl (mg/rat/day)	No. rats	Per cent mean tumor wt*	No. rats	Per cent mean tumor wt.*	Difference / S.E. diff.
400–600	70	100±4.05	65	73.3 ±3.05	5.27
200–400	60	100±4.16	59	90.55±3.56	1.7

* Mean percentages expressed with standard errors.

Sixty male Holtzman rats (mean weight, 204 gm.) were given inoculations by trocar in the left axilla of fragments of Walker tumor 256. Ten of these selected at random were treated for the next 10 days with intraperitoneal injections of 200 mg 2DG/rat/day on a rather rigid schedule of a 50-mg. dose every 6 hours. At 10 days the tumors of all animals were measured in three axes by caliper, except for the smallest ones, which could only be estimated. Of the 50 nontreated animals whose tumors were measured at this time, 38 were chosen on the basis of a good distribution about the mean (Chart 1). These were carefully paired for tumor size, half were then treated with 2DG, and the corresponding paired animals received an equivalent amount of glucose on the same injection schedule. At intervals during the growth period, measurements of tumor were made, and the mean tumor diameter in mm. was calculated and plotted. Mean tumor weights are expressed with standard deviations.

Thus there were five groups:

A. Ten animals treated with 2DG, days 1–10 and 18–20, inclusive.
B. Fifty rats untreated during the first 10 days. From group B, the following were selected on day 10:
   C. Eight untreated animals with small tumors used for comparison with group A during the following 13-day period.
   D. Nineteen animals treated with 2DG, days 11–17 and 21–23, inclusive.
   E. Nineteen controls matched with group D, receiving glucose on same schedule as D.

At 10 days the mean tumor diameter of the untreated group B was 8.2 mm. For the treated group A, it was 2.6 mm. It will be seen from Chart 2 that, on cessation of treatment, after a brief lag tumors of this group achieved a rate of growth approximating that of group C, which in turn closely parallels that of the other control group, E. When treatment was re-instituted in group A, this growth curve broke rather sharply away from the slope of the untreated animals and from its own immediately preceding rate of growth. After cessation of treatment the slope of growth again closely approached that of the untreated controls (Group C). At the termination of the experiment on day 24, the weights of the tumors of group A averaged 16.6 ± 5.0 gm, while those of group C averaged 30.6 ± 5.3 gm. This difference is 4.7 times the standard error of the difference.

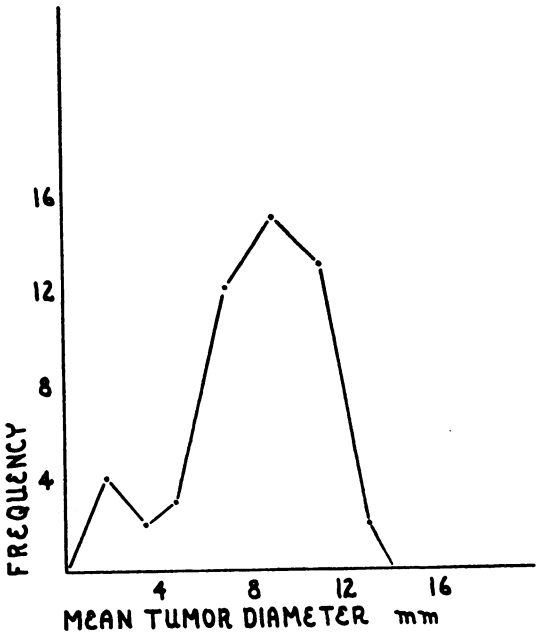

CHART 1.—Distribution data of tumor size on day 10 for 50 untreated animals.

The mean diameter of the 38 tumors divided into groups D and E was 9.2 mm. on day 10. After 7 days of treatment the mean tumor diameter of group D was significantly smaller than that of control group E; this difference persisted during the ensuing 3-day nontreatment period. During this nontreatment period the rate of growth of tumors in group D approached but did not equal that in group E. When treatment was re-instituted, tumor growth in group D decreased abruptly to that of the former treatment period (Chart 3).

The tumors of group E, when dissected and weighed 24 days following inoculation, gave a mean value of 40.4 ± 7.7 gm. The tumors of the treated group D had a mean weight of 20.5 ± 4.8 gm. The difference in mean tumor weight between these groups is 9.0 times the standard error of the difference.

The mean carcass weight of group E during the tumor growth period increased by 28 gm. The mean carcass weight of group D remained the same. This seems to indicate that, during the tumor growth period, no marked interference in general metabolism occurred. The treated animals at all times exhibited normal behavior. There was perhaps a slight increase in thirst after 2DG administration and a questionable tendency to spend slightly more time in the resting state. A bluish cast to the scrotal skin was independently noted by two observers, but the ears showed no evidence of cyanosis.

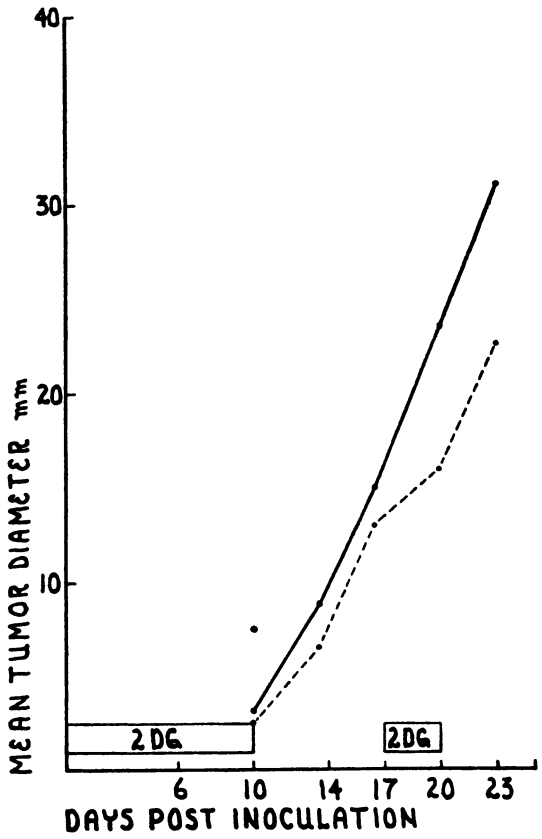

CHART 2.—Growth rate curves. Group C (untreated), solid line; group A (treated), broken line; isolated point at day 10 represents mean tumor diameter of group B.

At the time of sacrifice, the peritoneal cavities of the treated group, 2½ hours after the last intraperitoneal injection, contained an accumulation of fluid (2–4 ml.). The animals receiving glucose

showed no such effect. This probably indicated a somewhat slower absorption of the antimetabolite.

Statistical treatment of data relating to increment in mean tumor diameter for the different periods shows a significant change between the 2DG treatment and nontreatment periods (Table 3).

While nine of the 50 tumors in the 2DG experiment fell in the low range of tumor size at day 10, it seems highly improbable that the ten selected at random from the original 60 and treated with 2DG for the first 10 days could have had such a unique distribution as to fall consistently in this same range on the basis of chance. The general uniformity of tumor size within group A argues against this possibility and in the direction of a response to some inhibiting mechanism which prevented tumor growth during the latent period or immediately thereafter. The subsequent rapid growth of tumors of this group and the later response to 3 days of treatment, as well as the suppression of previously rapidly growing tumors of group D, also supports the strong probability that the difference between treated and untreated groups at day 10 was a result of the administered 2DG.

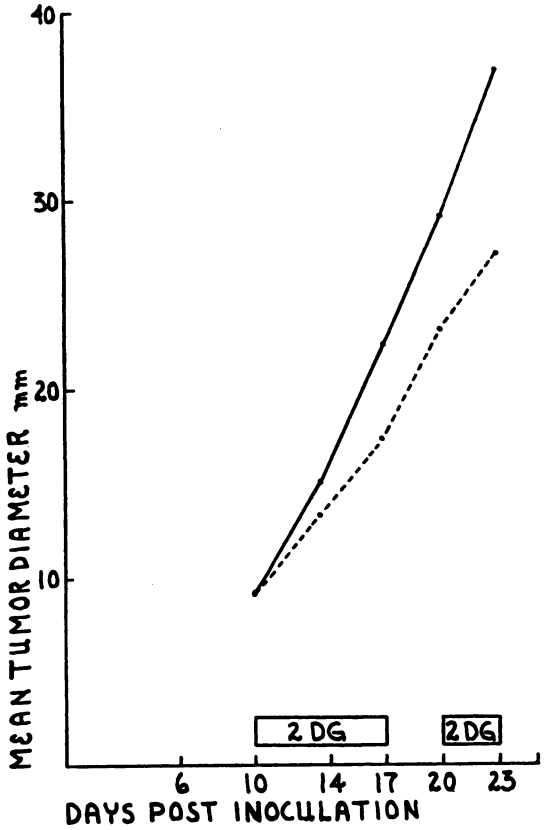

CHART 3.—Growth rate curves. Group D, treated, broken line; group E, matched controls on day 10, solid line.

The differences in mean tumor weight between each treated group and its corresponding control group are the more striking in view of the observations by one of us[1] that 2DG is rapidly eliminated through the kidney, 33 per cent of the activity of 2DG-C^{14} being in the urine 2 hours following intraperitoneal injection. It thus appears that only a portion of the amounts administered in this experiment can be considered to be related to tumor growth inhibition.

If a latent period of 5 days is arbitrarily subtracted from the total time span from tumor inoculation to sacrifice, the period of active tumor increase covers a period of 19 days. Group A was treated intermittently for 13 days of this period and group D for a total of 10 days, with tumors in both instances closely approximating one-half the weight of the corresponding controls. It thus appears that, whether the tumor is treated early or late, a semiquantitative effect is produced by a given amount of 2DG as used in these studies. This again supports the hypothesis that the glucose antimetabolites interfere with a process required continuously for the multiplication and growth of cells.

DISCUSSION

Both glucosamine and 2DG interfered significantly with tumor growth when given in multiple daily doses at the levels used here. Since both com-

[1] Wick, unpublished results.

TABLE 3

TUMOR GROWTH AS RATE PER DAY EXPRESSED AS INCREMENT OF MEAN TUMOR DIAMETER IN MM., WITH STANDARD ERROR

Tenth to 23d days inclusive; treatment periods italicized.

	First period (Days 10–14)	Second period (Days 15–17)	Third period (Days 18–20)	Fourth period (Days 21–23)	Mean
Group A	1.2±.21	2.1±.31	*0.9±.18*	2.4±.12	*1.90±.21
Group C	1.6±.38	2.0±.16	2.4±.07	2.7±.14	2.17±.19
Group D	*1.2±.05*	*1.3±.06*	1.7±.09	*1.3±.09*	*1.27±.06
Group E	1.7±.09	2.4±.07	2.1±.09	2.4±.11	2.15±.09

* Does not include third period.

$\frac{\text{Diff.}}{\text{S.E. Diff.}}$ = 3.6 for Mean group A against treated third period.

$\frac{\text{Diff.}}{\text{S.E. Diff.}}$ = 4.0 for Mean group D against nontreated third period.

pounds are rapidly excreted in the urine, however, only a portion of the amounts administered can be considered to be related to tumor growth inhibition.

The negative results which have been obtained with glucosamine by a number of workers may well be due to the even greater inefficiency of utilization when a single daily dose is given.

The effects of 2DG were greater and more consistent that those of glucosamine. This is in accord with their relative effectiveness as glucose antimetabolites (18, 19) and favors the hypothesis that the influence observed here is on glucose metabolism and only indirectly on the growth processes in the tumor cells.

The rapidity with which tumor growth increases toward the control rate after cessation of 2DG treatment also indicates that only a temporary condition has been imposed. This, together with the work reported by Migliarese and Bly (9), suggests that the Walker tumor grows rather consistently to the maximum capacity of the metabolic environment of its host and that it is this metabolic environment which has been temporarily modified by the administration of the glucose antimetabolites. The basic abnormality in the tumor cells does not appear to have been changed.

While these experiments demonstrate that tumor growth can be differentially affected by compounds which interfere with the utilization of glucose through the glycolytic cycle, the results may have little significance therapeutically, since the malignant cells do not seem to be permanently altered. It might be possible, however, to find an antimetabolite which would remain sufficiently long in the environment to cause death of the tumor cells. A reduction in energy level of tumor cell metabolism by this means might make such growths more susceptible to other compounds known to have an antitumor action.

SUMMARY

1. The intraperitoneal, divided-dose administration of glucosamine hydrochloride, 400–600 mg/rat/day (approximates 2,000–3,000 mg/kg/day) or 2-deoxy-D-glucose 200 mg/rat/day (approximates 1,000 mg/kg/day) exerted an antitumor effect in rats bearing Walker 256 tumor, without seriously compromising the general well being of the host.

2. The antitumor effect of 2DG was somewhat greater than that of glucosamine at ½–⅓ the glucosamine dosage level.

3. Changes in *rate* of tumor growth between treatment and nontreatment periods were significant when 2DG was used.

4. The modified metabolic environment created by the presence of 2DG was rather quickly changed after cessation of administration, with resumption of tumor growth to a rate approaching that of controls.

REFERENCES

1. BOAS, N. F., and FOLEY, J. B. Fate of Intravenously Administered Glucosamine in the Rat. Proc. Soc. Exper. Biol. & Med., 88:454–57, 1955.
2. CLARKE, D. A. Mouse Sarcoma 180. Cancer Research, Suppl. No. 3, pp. 14–17, 1955.
3. CRAMER, F. B. A Procedure for the Preparation of 2-Desoxy-D-glucose, J. Franklin Inst., 253:277–80, 1952.
4. CREECH, H. J.; HAUSCHKA, T. S.; HANKWITZ, R. F., JR., LITTLETON, B. J.; and ANDRE, J. Studies with Four Distinct Lines of the Ehrlich Ascites Tumor for the Evaluation of Twenty-seven Potential Inhibitors. Cancer Research, Suppl. No. 3, pp. 47–51, 1955.
5. ELY, J. O. 2-Deoxy-D-Glucose as an Inhibitor of Cancerous Growth in Animals. J. Franklin Inst., 258:157–60, 1954.
6. GELLHORN, A.; KELLS, A.; and GOLINO, M. Mammary Adenocarcinoma 755, Glioma 26, and Brown-Pearce Carcinoma. Cancer Research, Suppl. No. 3, pp. 38–43, 1955.
7. MALEY, F., and LARDY, H. A. Synthesis of N-substituted Glucosamines and Their Effect on Hexokinase. J. Biol. Chem., 214:765–73, 1955.
8. MARLOW, A. A., and BARTLETT, G. R. Glucosamine and Leukemia. Proc. Soc. Exper. Biol. & Med., 84:41–43, 1953.
9. MIGLIARESE, J. F., and BLY, C. G. The Determination of Maximum Tumor Growth Rates in the Rat. Cancer Research, 16:128–34, 1956.
10. QUASTEL, J. H., and CANTERO, A. Inhibition of Tumor Growth by D-glucosamine. Nature, 171:252–54, 1953.
11. RHOADS, C. P. Relationship of Nucleic Acid Anabolism to the Treatment of Cancer. M. Clin. N.A., 40:923–33, 1956.
12. SCHOLLER, J.; PHILIPS, F. S.; and BITTNER, J. J. Assays with First or Second Generation Transplants of Spontaneous Mammary Adenocarcinomas of Mice. Cancer Research, Suppl. No. 3, pp. 32–37, 1955.
13. SKIPPER, H. E., and THOMSON, J. R. Effects of a Series of Tumor-inhibiting Agents and Related Compounds on L1210 Leukemia and Drug Resistant Lines Thereof. Cancer Research, Suppl. No. 3, pp. 44–46, 1955.
14. SOKOLOFF, B.; EDDY, W. H.; SAELHOF, C. C.; and BEACH, J. Glucose Antagonists in Experimental Cancer. Arch. Path., 59:729–32, 1955.
15. SUGIURA, K. Merits of Ascites Tumors for Chemotherapeutic Screening. Ann. N.Y. Acad. Sc., 63:962–72, 1956.
16. ———. Studies in a Spectrum of Mouse and Rat Tumors. Cancer Research, Suppl. No. 3, pp. 18–27, 1955.
17. TARNOWSKI, G. S. Tumor Inhibition Studies on RC Mammary Adenocarcinoma. Cancer Research, Suppl. No. 3, pp. 28–31, 1955.
18. WICK, A. N.; DRURY, D. R.; and MORITA, T. N. 2-Deoxy-D-glucose, a Metabolic Block for Glucose. Proc. Soc. Exper. Biol. & Med., 89:579–82, 1955.
19. WICK, A. N.; DRURY, D. R.; NAKADA, H. I.; BARNETT, H. N.; and MORITA, T. N. Glucosamine and the Action of Insulin. J. Biol. Chem., 213:907–11, 1955.
20. WOODWARD, G. E., and CRAMER, F. B. 2-Desoxy-D glucose as an Inhibitor of Anaerobic Glycolysis in Tumor Tissue. J. Franklin Inst., 254:259–60, 1952.
21. WOODWARD, G. E., and HUDSON, M. T. The Effect of 2-Desoxy-D-glucose on Glycolysis and Respiration of Tumor and Normal Tissues. Cancer Research, 14:599–605, 1954.