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EFFECTS OF IRRADIATED GLUCOSE SOLUTIONS ON DMBA-INDUCED TUMORS IN RATS

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SUMMARY

This is a study of the effects of irradiated glucose-saline solutions (^{60}Co, 10^5 R at a dose rate of 1.7×10^6 R/h) on tumors induced by the intravenous injection of dimethylbenzanthracene (DMBA) in female Sprague-Dawley rats. Each animal received 10 cc intraperitoneal injections of the irradiated solution spaced over 17 days. As a control two groups of tumor bearing animals were used: one received irradiated isotonic saline solution, and the other unirradiated glucose-saline solution. It could be concluded that those animals which received the irradiated glucose-saline solution experienced retardation of the tumor disease both during and after the treatment period relative to the other groups under study. The results suggest that radiolysis of hexoses can give rise to a carcinostatic compound relatively non-toxic toward non-dividing cells.

A number of prior studies have shown that irradiated glucose solutions can produce disruptive effects upon mitotic processes in the cells of both plants and animals. Holsten *et al* [1] found that when plant cells were cultured in media which had been irradiated prior to adding the cells, effects were seen which resembled those caused by irradiation of the cells themselves. They also showed that the parent molecule of the radiation-generated toxicant was the glucose present in the culture medium. Similar studies by others [2-4] have shown that irradiated carbohydrates can produce a wide range of cytological aberrations in plant cells, including chromosomal stickiness and breakage, prophase arrest, and nuclear fragmentation.

Other studies have shown that effects of the same general kind are produced in animal cells by irradiated carbohydrate solutions. Shaw and Hayes [5] have studied the effects of irradiated sucrose on human chromosomes, and they observed stickiness, breaks, gaps and complex exchange abnormalities. Berry *et al* [6] had earlier shown that inhibition of growth of mammalian cells was produced by irradiated glucose and fructose in the culture media.

On the grounds that anti-mitotic agents have sometimes been found to have anti-tumor activity, it was decided to make a brief test of the action of irradiated glucose solutions against animal cancers. Preliminary experiments, which were only for *range-finding* purposes, consisted of injecting small groups of rats bearing chemically-induced tumors with dextrose-saline solutions irradiated to various dosages.

For these experiments, the test animals were rats of the Sprague-Dawley strain in which tumors had been induced by intravenous injection of dimethylbenzanthracene (DMBA). Each animal had at least one tumor with a maximum dimension of more than 0.5 cm. Test solutions were sterile glucose-saline solutions (5% glucose, 0.9% NaCl in H_2O) irradiated with ^{60}Co gamma radiation at a dose rate of 1.7×10^6 R/h.

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Animals were given two 10 cc intraperitoneal injections of the irradiated solution, spaced by eight days.

Solutions irradiated to 10^3 R and 10^4 R were without apparent effect upon the course of tumor growth, but in a group of six animals which received solutions irradiated to dose levels of 10^5 R there was seen a result that seemed to have possible significance. By 10 days after the second injection, three of the six animals had tumors that were much diminished in size. Inasmuch as the incidence of spontaneous regression generally observed in this tumor system is only about 3%, the 50% regression rate seen in this series, despite its small number, was regarded as justifying at least a modest further research effort.

It was therefore decided to undertake a small-scale, controlled study of the effects of irradiated glucose-saline solutions upon DMBA-induced tumors in rats. Due to practical limitations upon the size and scope of this work, definitive results were neither sought nor expected. Rather, this study was intended to serve as a basis for a decision as to whether to undertake a full-scale investigation in the future.

MATERIALS AND METHODS

To limit the number of variables in this experiment, only female Sprague-Dawley rats were used. Tumors were induced by a single injection of a lipid emulsion of dimethylbenzanthracene into the tail veins of 55-day old animals (1 cc of a 5 mg/cc concentration). Rats were held for a 10-week induction period during which most animals ($\sim$ 70%) developed at least one tumor in the skin or superficial connective tissue in a location where it could readily be measured. Animals were selected for the experiment which appeared to be in good general condition, and which had a tumor whose largest dimension was at least 0.5 cm but less than 1.0 cm. (Some of the rats selected displayed from the outset, or developed later, additional smaller tumors. In such cases, only the largest growth was used for observation and measurement).

Solutions were irradiated in a ^{60}Co facility which provided a dose rate of 1.7×10^6 R/h, so that teh desired dosage of 10^5 R was achieved in a 210-second exposure. In order that injections could be carried out as soon as possible after irradiation of the solutions, the animals were transported to a preparation room immediately adjacent to the irradiator and injected there.

The glucose-saline solution (hereinafter abbreviated G/S) and isotonic saline solution (I/S) were commercial products free of dissolved gases. All handling of solutions both before and after irradiation was carried out so as to prevent atmospheric contact, for the purpose of minimizing effects of dissolved O_2. Animals were premedicated with 2 mg intramuscular injections of phencyclidine hydrochloride to permit their easy handling during experiments. Test solutions were administered by careful intraperitoneal injection via 23-gauge needles.

There were 6 tumor-bearing rats in each of three groups as follows:

Group A — Each animal received four 10 cc injections of irradiated G/S solution spaced over 17 days.

Group B — Each animal received 10 cc injections of unirradiated G/S on the same schedule as Group A.

Group C — Each animal received 10 cc injections of irradiated I/S on the same schedule as Group A. This was a control group that checked for possible effects of irradiated saline solution without glucose.

To make it possible to follow the course of each tumor, rats were individually identified by ear notches. Starting at the time of the first treatment and continuing for

50 days or until death supervened, tumor dimensions were measured at approximately weekly intervals. Measurements were made of major and minor tumor axes and of tumor height. The product of these measurements was taken as proportional to tumor volume (the ellipsoidal approximation) and this number was plotted so that a continuous history of tumor growth was available for each animal. The rats were weighed and examined for general condition at the time of each measurement.

Animals which were found in obviously terminal condition were euthanized and the tumors were removed and fixed for possible future histological study.

The tumor measurements, calculations and data plotting were carried out by a *blind* operator, a specifically trained laboratory assistant who was the only person to handle the records and who was unaware of the group assignments of the animals. In similar fashion, the persons who carried out the animal treatments were unaware of the results of growth measurements, so that the study satisfied the general specifications for a double-blind experiment.

RESULTS

The results of these experiments are illustrated by the graphs, (Figures 1-3). Because of the limited number of animals in each group it was considered that statistical analysis of these data was inappropriate. We will therefore discuss our findings in terms of observable trends, subject to the qualification that, because of the small numbers involved, only the major features of the graphs have probable validity.

Figure 1 shows that Group A, the animals which received a series of four 10 cc injections of irradiated G/S, experienced a decrease in tumor volume to an average size that was about 80% of the original volume. The size decrease began shortly after the initial treatment and it continued in roughly linear fashion until several weeks after the last treatment, after which there was an apparent increase in average tumor volume.

Measurements of average body weight in Group A showed that there was no growth during the treatment period. This was quite possibly due to stressful factors coincident to treatment, such as transportation to and from the treatment room and the physical restraint of the animals that was needed to carry out the large-volume injections. The arrest of growht might also have been due in part to low-grade toxic effects of the irradiated solution. However, during the post- treatment or observation period the animals exhibited a positive average growth curve suggestive of relatively good general condition, an observation very much at variance with general experience with rats bearing DMBA — induced tumors. In the final phase of the period of observation, during which tumor growth resumed, there was a decline in average body weight, reflecting the terminal deterioration in the general condition of the animals.

Visual observation of Group A rats gave additional evidence of relatively good condition during the period of treatment and for several weeks thereafter. The pelage remaind sleek, and they were normally active. Although measurements of food intake were not made, by routine observation it was apparent that this group maintained a normal level of consumption.

The results of measurements upon Group B, the rats which received injections of unirradiated G/S, are depicted in the graph of figure 2. In this group the average tumor volume decreased during the treatment period, but only slightly. It is not known whether this represents an actual effect of the treatment or whether it is merely a statistical fluctuation. At any rate, immediately after the tratment period the tumor

growth curve became strongly positive in slope and remained so throughout the period of observation.

The averages of body weights of Group B indicated that no growth occurred during the treatment period. During the post-treatment observation period there occurred some increase in average weight due, no doubt, to the very large masses attained by most of the tumors.

Visual inspection of Group B animals gave the impression of poor general condition; the fur was rough and the animals were judged to be less than normally active.

Measurements upon the Group C animals, which received injections of irradiated saline solution, are plotted in the graph of figure 3. These animals exhibited a relatively monotonic increase in average tumor volume during the treatment and observation period. The upward trend of body weight was in all likelihood due to the increase of tumor mass at the expense of normal body mass, for the animals were increasingly cachectic. This, together with the poor condition of the pelage, suggested that the general condition of this group was poorer than that of the other groups.

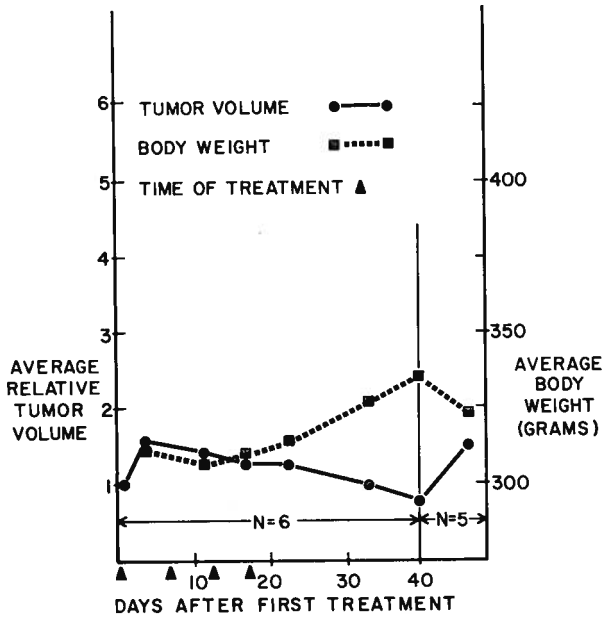

Fig. 1 — *Group A (treatment - irradiated G/S)*

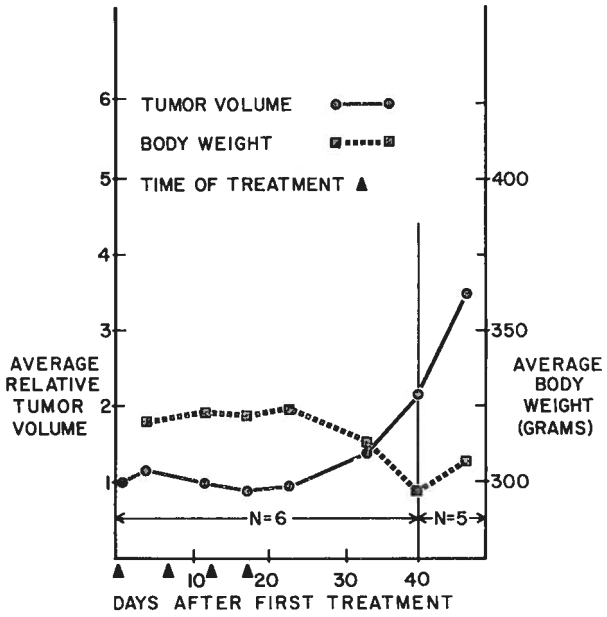

Fig. 2 — *Group B (treatment - unirradiaed G/S)*

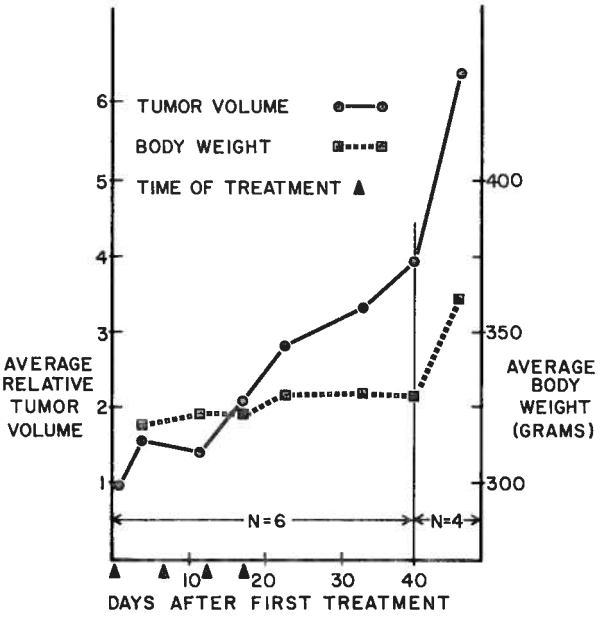

Fig. 3 — *Group C (treatment - irradiated saline)*

DISCUSSION AND CONCLUSIONS

Animals which received the irradiated G/S solutions experienced retardation of their tumor disease both during and after the treatment period, relative to the other groups in the study.

It appears that there was also some tumor-retardant effect of the unirradiated G/S solution, but this effect lasted only during the treatment, not afterward. It is possible that this was an *in vivo* Crabtree effect such as leads to activation of lysosomes, an efficient *cell suicide* mechanism.[7, 8] It was earlier reported that glucose infusions alone may cause regression of rat carcinosarcomas.[9]

It is concluded that radiation-generated H_2O_2 was not the anti-tumor agent in these experiments, for this would have had about the same concentration in irradiated saline as in irradiated glucose-saline.

The most reasonable explanation of our results is that glucose was the parent substance of a tumor-retardant product generated by irradiation of this sugar in aqueous solution. Much prior study has been applied to the radiolysis of glucose e. g.[10-12] Holtz and Becker[13, 14] showed that irradiated monosaccharide solutions developed strong negative redox potentials and maximal absorption at 265-290 nanometer. Swallow identified the dominant product of glucose irradiation as glucuronic acid, noting that at low O_2 concentrations, as in our experiments, glyoxal and glucuronic acid are produced. Glyceraldehyde is a minor product, possibly formed via an excitation process as in the UV photolysis of glucose.[15]

Hills and Berry[16] identified glyoxal as the sugar radiolysis product with the greatest potential for biological effects, showing that cytotoxic effects of irradiated carbohydrates could be imitated by glyoxal alone. Further attention was given to glyoxal by Szent-Gyorgyi *et al.*[17] They attributed an important cell—regulation function to a keto — aldehyde derivative of glyoxal which they believed to interact with the free sulfhydryls involved in cell division.[18, 19] This compound is said to inhibit mitosis without producing cell damage, and although it is present in all normal tissues it was absent from cancer cells, suggesting that it may have a basic relationship to neoplasia.[20]

In summary, earlier studies in conjunction with our limited results suggest that hexose radiolysis can produce a carcinostatic compound relatively harmless to non-dividing cells. It is of some interest to speculate on possible relationship between glucose radiolysis and the *abscopal* effects of radiation. This term refers to radiation actions at places distant from the directly irradiated site.[21] The concept originates from clinical experience,[22, 23] but it has been examined experimentally.[24-26] Some speculations about the compound responsible for abscopal effects have involved H_2O_2, but Mitchell[27] has argued against this connection because of differences between radiation effects and peroxide toxicity.

Although glucose is present in all tissues and thus could hypothetically be implicated in abscopal effects, the radiation doses used in clinical practice are much smaller than those used in *in vitro studies* of glucose radiolysis,, so the concentration of glucose radiation products from therapeutic radiation would be small. Under these conditions, abscopal effects could be expected only in neoplasms which happened to be highly sensitive to glucose radiolysis products.

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RESUMO

Estudou-se o efeito da injecção intraperitoneal de soluções irradiadas de glucose-soro fisiológico (^{60} Co, 10^5 R a uma taxa de dose de $1{,}7.10^6$ R/h) em tumores induzidos por injecções intravenosas de dimetilantraceno em ratos fêmeas Sprague-Dawley. Cada animal recebeu 10 cc da solução irradiada em injecções distribuidas por um período de 17 dias. Usaram-se dois grupos de controlo formados por animais portadores de tumores; um recebeu uma solução irradiada de soro fisiológico e o outro soluções de glucose em soro fisiológico não irradiadas. Observou-se nos animais que tinham recebido a solução de glucose irradiada que o tumor cresceu mais lentamente do que nos animais dos grupos de controlo tanto durante o período de tratamento como algum tempo depois. Os resultados sugerem que a radiólise de hexoses pode originar produtos carcinostáticos relativamente não tóxicos em relação às células não em divisão.

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