BIOCHEMICAL EFFECTS OF IRRADIATED SUGARS ON HUMAN CELLS IN CULTURE

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Pericles Markakis
Major professor

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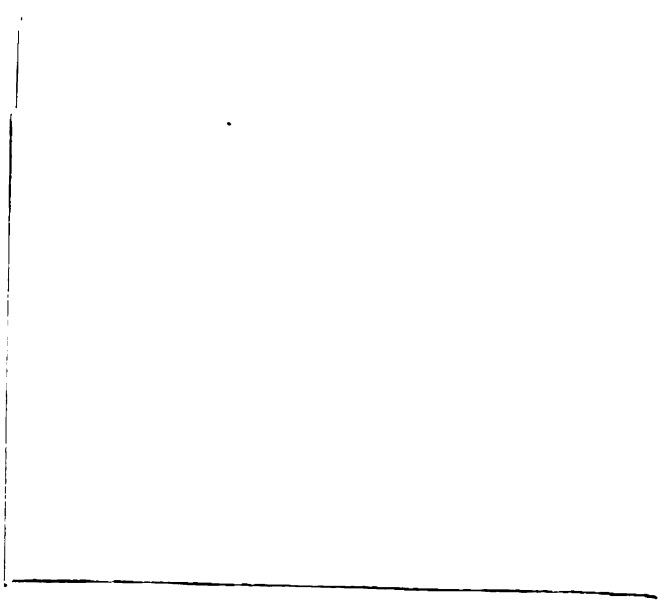

ABSTRACT

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By

Noemi Diaz-Santiago

In the present study two criteria were used to test the toxicity of gamma-irradiated sugar solutions on human amnion AV_3 cells in culture: a) the ability of the cells to grow and multiply after having been incubated with irradiated sucrose for various periods, and b) the effect of irradiated glucose, fructose and sucrose on the synthesis of DNA, RNA, and protein in the same cells.

Minimum essential medium (MEM) containing 5.8×10^{-2}M of 3 Mrad irradiated sucrose was incubated with AV_3 cells for 6, 12 and 24 hours at 37° C. After these periods the medium containing irradiated sucrose was replaced by medium containing non-irradiated sucrose and cell counts were made at 2, 4 and 6 days. The duration of the incubation of the cells with the irradiated sugar was critical for their growth and replication. The percentage of growth inhibition

for the incubation periods of 6, 12 and 24 hours were: 40, 60, and 90 respectively.

The synthesis of DNA, RNA and protein in human amnion cells was assessed by following the incorporation of tritiated precursors added to MEM in the presence or absence of 5.8×10^{-2}M sucrose, glucose or fructose solutions which had been irradiated at 1, 2 and 3 Mrad. For a period of 6 hours of incubation with the 3 Mrad irradiated sugars the following percentages of inhibition of DNA, RNA and protein synthesis (in that order) were: 49, 26 and 38 for sucrose; 20, 14 and 24 for glucose; and 51, 26 and 34 for fructose.

Other factors such as the effect of pH, the activity of DNA polymerase, the presence of catalase in the medium containing 3 Mrad irradiated sucrose and the time elapsed between irradiation of the sugar solution and testing for its effects on the DNA synthesis in AV_3 cells were also investigated.

A considerable reduction in the toxicity of irradiated glucose was observed by maintaining the pH of the MEM containing irradiated glucose medium at 7.2 during the incubation period with the cells.

The activity of DNA polymerase from an AV_3 cells extract was assessed following polymer formation measured by the incorporation of a nucleotide labeled with ^3H into acid-insoluble material

(presumably DNA). The enzyme was not affected by the presence of irradiated sucrose in the substrate.

It was also observed that a concentration of 40 μg/ml (66 units/ml) of catalase only partly eliminated the deleterious effects of 3 Mrad irradiated sucrose to human amnion cells.

Sucrose solutions irradiated at 3 and 4 Mrad and stored for seven months at room temperature did not show a reduction on the inhibitory effects on the synthesis of DNA in AV_3 cells.

Evidence was presented to show that the damage caused to human amnion cells by the radiolysis products of sucrose and fructose cannot be repaired. The duration of the incubation of the cells with the irradiated sugar, the radiation dose applied, the pH of the medium and the stage of development of the cells are critical for their survival. It seemed that only the fraction of the cell population specifically involved in DNA synthesis (the S phase of development) was severely affected by the radiolysis products of the sugars. This effect is time dependent: the longer the incubation period the higher the number of cells rendered unable to grow and multiply.

BIOCHEMICAL EFFECTS OF IRRADIATED SUGARS ON HUMAN CELLS IN CULTURE

By

Noemí Díaz-Santiago

A THESIS

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TO MY PARENTS

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INTRODUCTION

Studies on the feasibility of preserving foods by ionizing radiations were undertaken shortly after World War II. Early work in the United States demonstrated a number of possible industrial applications on a theoretical and laboratory basis (Brasch and Huber, 1947). An extensive program on the radiation sterilization of foods was started in 1953 by the U. S. Army Quartermaster Corps, which was attracted by the advantages of preserving food for long periods of time without the use of heat or refrigeration.

The fundamental requirement for obtaining Governmental approval of the application of irradiation to a given food is the production of evidence that such food is wholesome for human consumption. Although the recognized method of producing such evidence is by animal feeding trials, the results of studies using a less complicated "model system" should not be overlooked.

It is well established that ionizing radiation exerts biological effects not only by direct action on the organism, but also by changing the environment in which organisms live. Some of these indirect effects are toxic. While there are considerable

data on the cytotoxic and radiomimetic effects of irradiated culture media and sugar solutions, their precise biochemical nature is yet to be clarified.

In the present study two criteria were used to test the toxicity of gamma irradiated sugar solutions on human amnion AV_3 cells in culture: a) the ability of the cells to multiply after having been in contact with sucrose for various periods and, b) the synthesis of DNA, RNA, and protein in the same cells.

Other factors such as the pH of the irradiated solutions, the time elapsed between irradiation and testing, the duration of the exposure of the cells to irradiated sugar, and the effect of catalase on the irradiated media were also studied.

REVIEW OF THE LITERATURE

A number of studies have been conducted in which organisms were grown in the presence of irradiated substances. Such organisms included bacteria, yeasts, higher plants, the fruit fly, and mammalian cells.

The radiomimetic effects of irradiated media have been known for many years. As early as 1887, while investigating the killing of the spores of the anthrax bacillus by sunlight, Roux came to the conclusion that germination of the spores could be inhibited by materials which were formed as a result of the oxidation of the carbohydrate fraction of the medium (Blank and Arnold, 1935). Coblentz and Fulton (1924) and Woodrow, Bailey and Fulmer (1927) showed that the ability of a medium containing carbohydrates to support the growth of micro-organisms was diminshed if the medium was irradiated with ultraviolet energy previous to inoculation.

Blank and Kersten (1935) showed that modified extract agar could be so altered by the action of soft X-rays that it would no no longer support the growth of Bacillus subtilis. They concluded

that inhibition results from the formation of a toxic material in the agar, the major carbohydrate of the medium. This discovery led to the investigation of the action of radiation on other carbohydrates. Blank and Arnold (1935) observed that Bacillus subtilis could be similarly inhibited by the addition to the culture medium of an irradiated solution of any one of 20 different carbohydrates and 3 carbohydrate derivatives. Baumgartner (1936) confirmed the former workers' observation and also stated that such radiation of carbohydrates is accompanied by marked production of acid, mainly formic acid. He also observed that neutralization of this acidity restores the ability of the culture media to support growth of the bacteria.

Stone, Wyss and Haas (1947) obtained initial evidence that ultraviolet light produced chemical mutagens in bacterial media capable of inducing mutations in unirradiated organisms placed in these media.

In the past few years, several articles dealing with cell growth and chromosomal aberrations induced by irradiated media have been published. Molin and Ehrenberg (1946) observed a bacteriostatic action exerted by irradiated glucose solutions on Pseudomonas species. An effect on the incorporation of uracil or thymine in the cells of Escherichia coli as a response to irradiated

medium was reported by Pollard et al. (1965). The inhibitory action of irradiated sucrose solutions on Salmonella typhimurium was studied by Schubert and Watson (1969). They attributed this effect to hydroxyalkyl peroxides and hydroperoxides resulting from sugar hydrolysis.

Cytological aberrations in higher plants produced by irradiated carbohydrates have been reported by several investigators.

Holsten et al. (1965) found that irradiated sucrose solution and certain fractions derived therefrom inhibit the growth of carrot cells and Vicia faba root cells, leading to chromosomal aberrations and impairment of cell division. Chopra et al. (1963) reported chromosome breakage in barley and onion seeds grown on orange and apple juice which had been irradiated with 200 Krad of gamma rays. A nucleotoxic effect due to irradiated glucose on onion and barley root tip cells was observed by Moutschen and Matagne (1965). The inhibitory effects of irradiated sucrose on the germination and growth of pollen of Tropaeolum majus was reported by Kesavan and Swaminathan (1967). Bajaj (1970) studied the effects of irradiated (2 Mrad) sucrose, glucose and fructose on the growth and development of callus tissue cultures, excised roots, ovules and embryos of Glycine max, Nicotiana tabacum, Pelargonium hortorum and

Phaseolus vulgaris. He observed growth inhibition by 30 to 50%. The growth of immature ovules and embryos of Nicotiana tabacum and Phaseolus vulgaris, respectively, was consideraly inhibited by irradiated sucrose. However, this growth inhibition was markedly reduced in the roots of Glycine max, if these roots were grown on an irradiated glucose medium stored for six months.

Several investigators, Swaminathan et al. (1963), Rinehart and Ratly (1965), and Prakash (1965), have demonstrated an increase in mutations when Drosophila melanogaster are reared on irradiated foods. Nevertheless, these results failed to be reproduced by other workers (Chopra, 1965; Khan and Alderson, 1965; and Reddi et al., 1965).

The cytotoxic effects of irradiated culture media containing carbohydrate (glucose and fructose) to human and animal cells were first observed by Berry and co-workers (1965), who studied the reproductive survival of mammalian cells. They attributed the toxicity to the production of glyoxal in the solution. Chromosome aberrations were observed when human lymphocytes were exposed to irradiated sucrose (Shaw and Hayes, 1966). Kesavan and Swaminathan reported cytotoxic effects of irradiated culture medium on human leukocytes. Scott et al. (1966) observed growth inhibition of L5178 Y lymphoma cells when these were in contact with irradiated

medium. Kellner and Kaindl (1967) studied the influence of glyoxal on the growth of human fibroblasts. They found that glyoxal could be degraded by the cells and thus no toxicity was apparent after 24 hours of contact between cells and the glyoxal containing medium.

De, Aiyar and Sreenivasan (1969) investigated the effect of irradiated sucrose on rats. When rat liver slices were exposed to irradiated (0.5 Mrad) sucrose solutions, inhibition of succinate oxidation and phosphorylation as well as the synthesis of lipids, proteins and DNA was observed. However, no deleterious effects could be observed in the 12 rats fed irradiated sucrose solutions for a period of 8 weeks. Articles, books and monographs dealing with the effects of irradiated media on living organisms have been published. These include the proceedings of a symposium on implications of organic peroxides in radiobiology (Feenstein, 1962), a review article by Stone (1955) on the general effects of medium irradiation on genetic material, a review by Scarascia-Mugnozza et al. on the genetic effects produced by irradiated food and food components (1965), and Latarjet's discussion on the viral and bacterial effects using growth as a criterion (1956).

The products of radiolysis of carbohydrates have been studied in great detail by Phillips and co-workers (1965, 1969). Their work has served as the basis for many of the studies referred to in this manuscript.

MATERIALS AND METHODS

Culture

Human amnion AV_3 cells obtained from the American Type Culture Collection (Rockville, Md.) were cultured to form a monolayer in Roux bottles at 37° C in a humidifed 5% CO_2 atmosphere in Eagle's Minimum Essential Medium (MEM) supplemented with 10% calf serum. Fifty ml of MEM were used per bottle of culture. After several days of incubation a monolayer of cells was formed, the growth medium was discarded, and 10 ml of 0.05% trypsin preparation in Puck's Saline "A" was added to the culture bottle in order to detach the cells from the glass. The cells collected from several bottles were pooled in a flask containing MEM and their concentration was determined with a hemocytometer. Falcon disposable plastic tissue culture plates (60 mm in diameter) were used to inoculate media for the experiments. Equal amounts of cells were pipeted into each plate (2×10^5 cells/plate). For any given experiment the cells were incubated for at least 18 hours at 37° C to allow the cells to attach before any treatment was applied. The pH of the culture medium was 7.2.

Irradiation

Sugar solutions containing 4% of either glucose or fructose or sucrose were prepared in distilled-demineralized water and filter sterilized using Millipore filters, HA - 0.45μ. The solutions were transferred into sterile 25 ml vials, screw capped and placed at the calculated distance from the ^{60}Co source in order to absorb the desired dose. The 50,000 Ci cobalt-60 irradiator installed in July 1967 in the Department of Food Science of this University was used for all the experiments described in this work. Irradiations were performed at constant temperature (20° C) for a period of 16 hours.

Labeled Compounds

Tritiated thymidine (^{3}H - Tdr), tritiated uridine (^{3}H - Udr), and tritiated leucine (^{3}H - leucine), specific activity 2.0 Ci/mM, were obtained from New England Nuclear. ^{3}H - Tdr was used as a labeled precursor for DNA synthesis, ^{3}H - Udr for RNA synthesis, and ^{3}H - leucine for protein synthesis in AV_3 cells incubated with MEM containing irradiated sugars.

DNA, RNA, and
Protein Synthesis

Measurement of DNA synthesis was performed by following the incorporation of tritiated thymidine into trichloroacetic acid (TCA)-insoluble material as described by Chu and Regan (1966). ^{3}H-thymidine, specific activity 2.0 Ci/mM, 2μCi/ml in MEM-sucrose was used for the experiments.

Three ml of MEM containing ^{3}H-Tdr and 5.8×10^{-2}M irradiated sugar were added to culture plates of AV_3 cells in a monolayer. The plates were incubated at 37° C for 2, 4, and 6 hours to allow for the incorporation of the labeled precursor into DNA. At the end of each period the plates were placed over ice to stop the incorporation of thymidine. The MEM containing ^{3}H-Tdr was discarded and replaced by 3 ml of Puck's Saline D solution. The monolayered cells were disrupted by sonication for 10 seconds with a Branson 140C sonnifier and 0.1 ml of well-agitated sonicate was applied to each of two Whatman 3 MM 2.3 cm diameter filter discs. After being washed three times in cold 5% TCA, twice in absolute ethanol and once in acetone, the discs were dried and placed in liquid scintillation vials. Five ml of a solution containing 4g of 2,5'-diphenyloxazole (PPO) and 0.1g of 1,4-bis[2-(4-methyl-5-phenyloxazole)] benzene (dimethyl POPOP) per liter of toluene were

used. The radioactivity was measured in a Packard Tricarb Scintillation Spectrometer. Each data point consists of the average of the counts per minute (cpm) from duplicate plates and duplicate samples from each plate. A similar procedure was used to determine RNA and protein synthesis in AV_3 cells, only the labeled precursors were changed. Unirradiated sugar controls were run simultaneously with each treated sample throughout all the experiments described in this work. The pH of the media was kept at 7.2 unless otherwise stated.

A series of experiments was conducted in order to study the effect of irradiated glucose, fructose and sucrose on the DNA, RNA and protein synthesis of human amnion (AV_3) cells in culture. The radiation doses used were 1, 2, and 3 Mrad. These doses were chosen because it was considered desirable to work within levels below those recommended for the complete radiation sterilization of foods. The molar concentration of the sugar solutions added to the growth media was 5.8×10^{-2}M. This concentration was used for all the studies reported in this manuscript. The duration of the period of incorporation of the particular labeled precursor (thymidine, uridine or leucine) was 6 hours.

Cell Viability Study

Tissue culture plates containing 1.5×10^6 cells/plate were pre-incubated to form a monolayer in 2% sucrose-MEM. The medium was discarded and 3 ml of 2% 3 Mrad irradiated sucrose-MEM at pH 7.2 were added to the monolayered cells. The plates were then incubated at 37° C for 6, 12 and 24 hours. The MEM containing unirradiated 2% sucrose was added to the control plates. After each incubation period the media were removed from the plates, new MEM-2% unirradiated sucrose was added, and the plates were placed back in the incubator. Cell counts were made with a hemocytometer at 2, 4 and 6 days after the removal of the irradiated sucrose medium.

Activated Carbon

Activated carbon was used to try to remove the slight yellow pigment and possibly toxic fraction of the irradiated sugars. 2.4g of activated carbon were added to 60 ml of 4% solutions of sucrose irradiated at 1, 2 and 3 Mrad. This amount of carbon represents a 1:1 proportion by weight with the irradiated sugar. The mixtures were continuously agitated in 250 ml conic flasks for one-half hour. Filtration through Whatman No. 1 paper was done to remove the carbon. The clear filtrate of the 4% irradiated sucrose

was then filter-sterilized through a Millipore HA-0.45μ filter and mixed with equal volumes of ^{3}H-Tdr-MEM 2μCi/ml. The incorporation of ^{3}H-Tdr into DNA of AV_3 cells was determined at 2, 4 and 6 hours of incubation at 37° C with the monolayered cells.

DNA Polymerase Activity

DNA polymerase was obtained from an AV_3 cells extract. The cells were grown in Roux bottles until a monolayer was formed. They were harvested using 5 ml of 0.02% EDTA per bottle of culture. A pellet was collected in a 15 ml centrifuge tube and 5 ml of 10 mM Tris buffer at pH 8.1 and 1 mM in $MgCl_2$ were added to each tube. The pellet was reduced to a suspension by shaking, and the suspension was allowed to stand for 1 hour at 37° C. The cells were spun at 6,000 rpm in a refrigerated International Electric Centrifuge, and the supernatant was called extract #1. Extract #1 was centrifuged for 1 hour in a Sorval high speed refrigerated centrifuge at 30,000 rpm to collect extract #2. This was the extract used for the DNA polymerase activity assay. The DNA polymerase activity assay was conducted according to the method described by Zimmerman (1966). The requirements for maximal activity of the enzyme system which has been called DNA polymerase are the presence of all the four deoxyribonucleoside-5'-triphosphates, Mg ions and DNA primer.

Catalase Activity

Stock solutions of catalase were prepared from beef liver catalase solution obtained from Sigma Chemical Company, Inc. (33,000 Sigma units/ml or 20 mg/ml). One unit decomposes 1μ mole of hydrogen peroxide per minute at pH 7.0 at 25° C. Two concentrations were used for the experiments: 20μg/ml and 40μg/ml. These concentrations of catalase were tested in two experiments in which the catalase was incubated at 25° C at pH 7.0 with 3 Mrad irradiated 5.8×10^{-2}M sucrose solution from 20 minutes to 3 hours. MEM containing unirradiated sucrose was used as control as well as heat inactivated catalase. At the end of the catalase incubation period ^{3}H-Tdr MEM was mixed, 1:1 by volume, and the synthesis of DNA in AV_3 cells was measured by following the incorporation of the DNA labeled precursor into TCA-insoluble material for a period of 4 hours. Each count recorded represents the average of duplicate counts of duplicate samples.

Irradiated Sucrose in Storage

Sucrose solutions prepared 4% by weight in distilled-demineralized water were filter-sterilized and transferred with a sterile syringe into 20 ml sterile ampules. The ampules were sealed and placed at the calculated distances from the ^{60}Co source

to absorb 3 and 4 Mrad respectively. The irradiated solutions were kept at room temperature until they were assayed for toxicity on AV_3 cells. The effect of the presence of 3 and 4 Mrad irradiated sucrose on the synthesis of DNA in AV_3 cells was determined at 0, 2, 4 and 7 months of storage.

RESULTS AND DISCUSSION

A. Cell Viability

The ability of human amnion AV_3 cells to multiply after having been in contact with irradiated sucrose was tested.

AV_3 cells in their exponential growth phase were transferred to minimum essential medium (MEM) containing 2% sucrose irradiated at 3 Mrad. The system was incubated at 37° C for 6, 12 and 24 hours. After these periods the medium containing irradiated sucrose was replaced by medium containing non-irradiated sucrose and cell counts were made with a hemocytometer at 2, 4 and 6 days from the time of removal of the irradiated sucrose. The results are presented in Figure 1.

The growth inhibitory action of 3 Mrad irradiated sucrose is time dependent. The findings suggest that the damage caused to the cells by the presence of radiolysis products of sucrose is not repaired even after removal of such compounds from the growth medium.

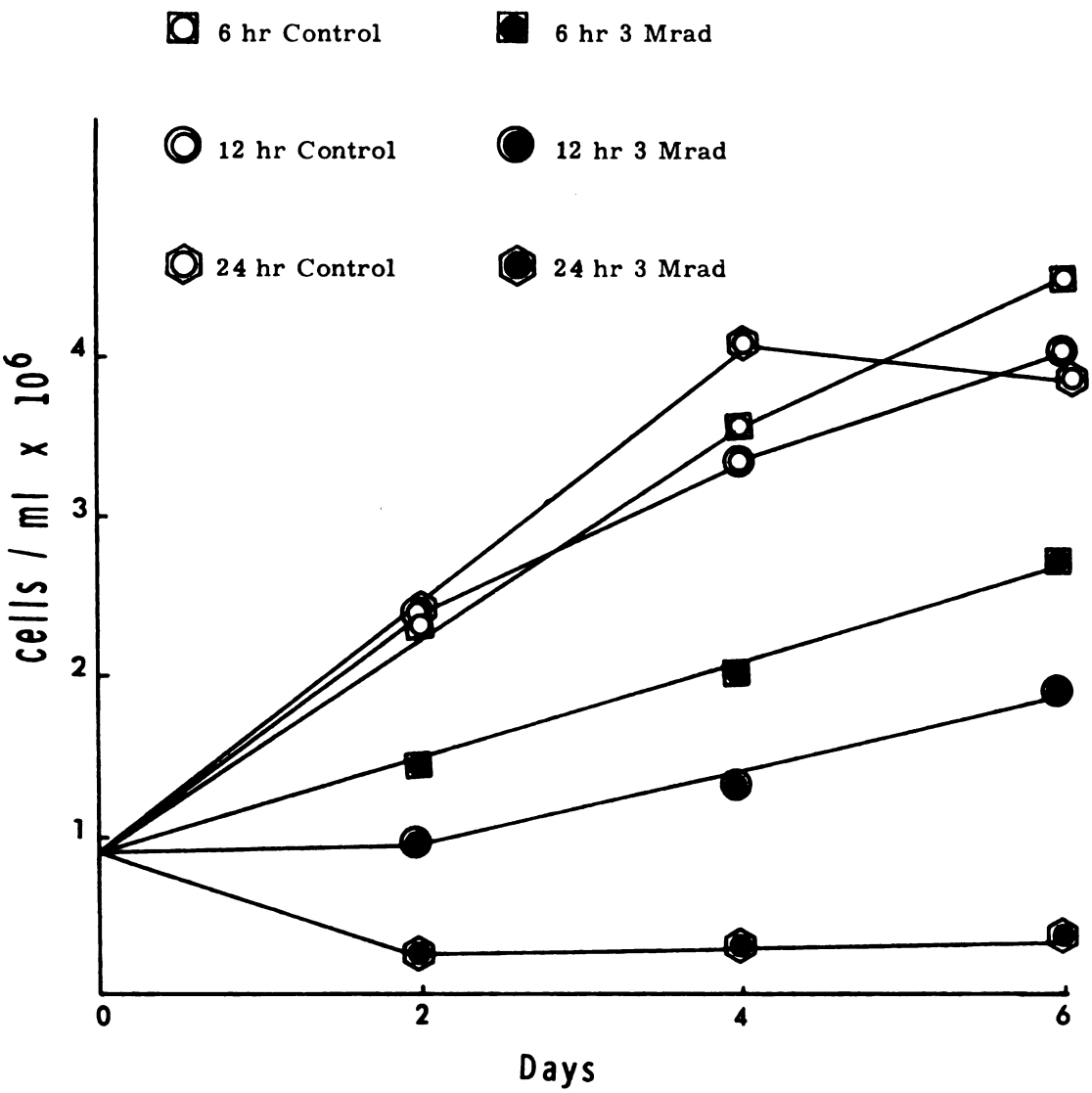

Figure 1. --Effect of duration of incubation on the growth of AV_3 cells exposed to 3 Mrad irradiated sucrose solution 5.8×10^{-2}M in MEM.

B. Synthesis of DNA

According to Edmunds (1964) the doubling of DNA is not the only requirement for cell division, but inhibition of DNA synthesis will generally result in an inhibition of cell division.

A series of experiments was performed in order to study the effects of irradiated glucose, fructose and sucrose solutions on the synthesis of DNA in AV_3 cells.

1. Effects of irradiated water and dose rate

Since all the sugar solutions used for this study were prepared with distilled and demineralized water, it was important to determine if there is an effect on the DNA synthesis in AV_3 cells due to the radiolysis products of water. The results presented in Figure 2 show no difference between the water irradiated at 3 Mrad and the unirradiated water. Two dose rates were used in this experiment: 187 Krad/hr for 16 hours and 1574 Krad/hr for 1 hour and 54 minutes. These were found not different from the control (Figure 2).

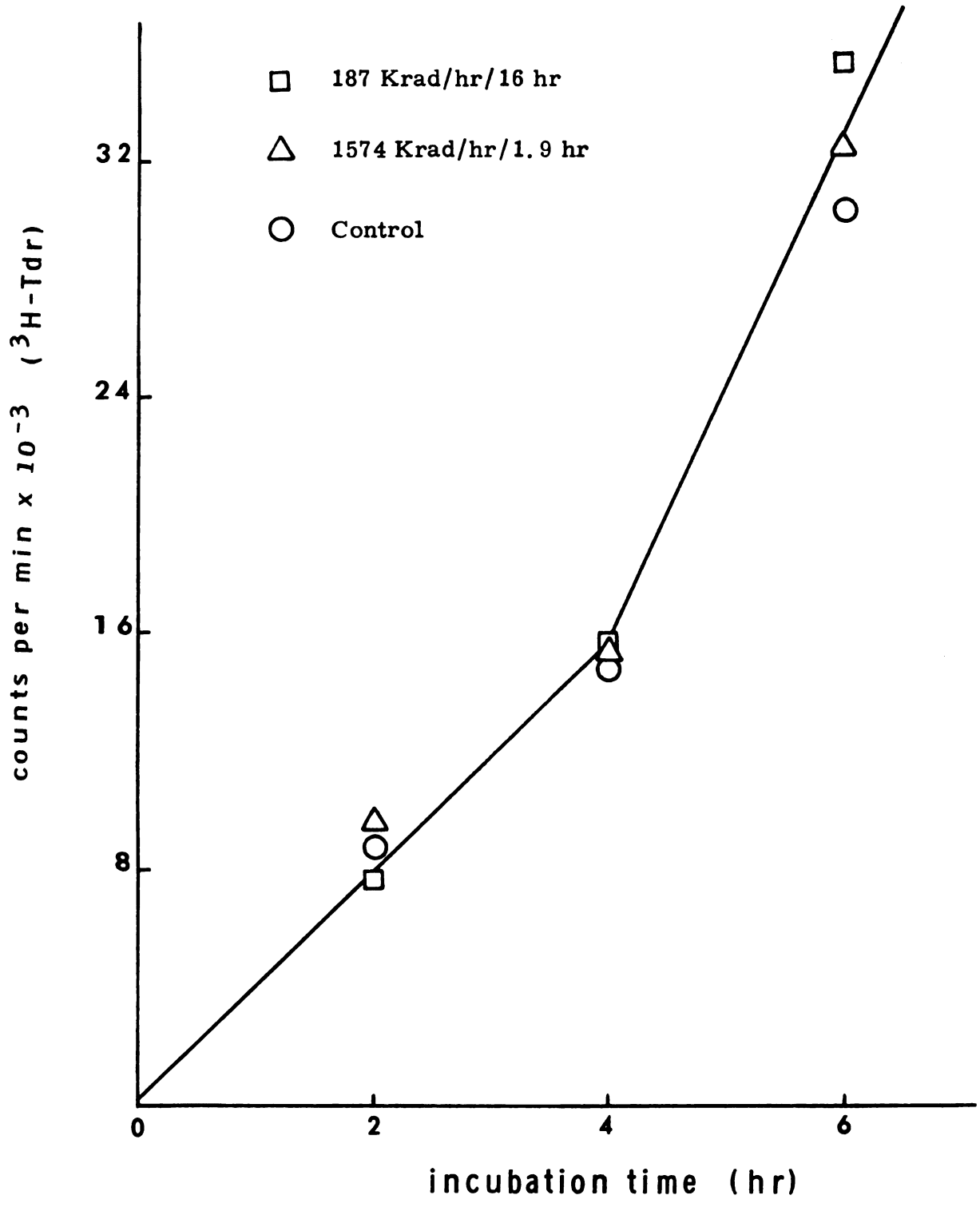

Figure 2. --Effect of water irradiated at different dose rates on the ^{3}H-thymidine uptake in DNA synthesis by AV_3 cells.

2. Effect of irradiated sucrose on DNA synthesis--preliminary experiment

A preliminary test on the synthesis of DNA was done on AV_3 cells in contact with 2% sucrose-MEM for 2, 4 and 6 hours using irradiation doses of 0, 1, 2 and 3 Mrad (Figure 3). Inhibition of DNA synthesis was most marked at 3 Mrad.

3. The effect of activated carbon

An attempt to remove the toxic compounds from the irradiated sucrose solutions was made by using activated carbon. A 13% increase in the DNA synthesis of the cells was observed for the 1 Mrad treated sucrose (Figure 4). No significant difference was found between the 2 and 3 Mrad carbon treated samples and the nontreated ones. Thus activated carbon does not seem to be effective in removing the compounds responsible for the toxicity of irradiated sucrose.

The effects of 3 and 4 Mrad irradiated 2% glucose or fructose in MEM medium on the DNA synthesis of AV_3 cells were compared to nonirradiated controls. A higher inhibition of DNA synthesis by fructose was observed (Figures 5

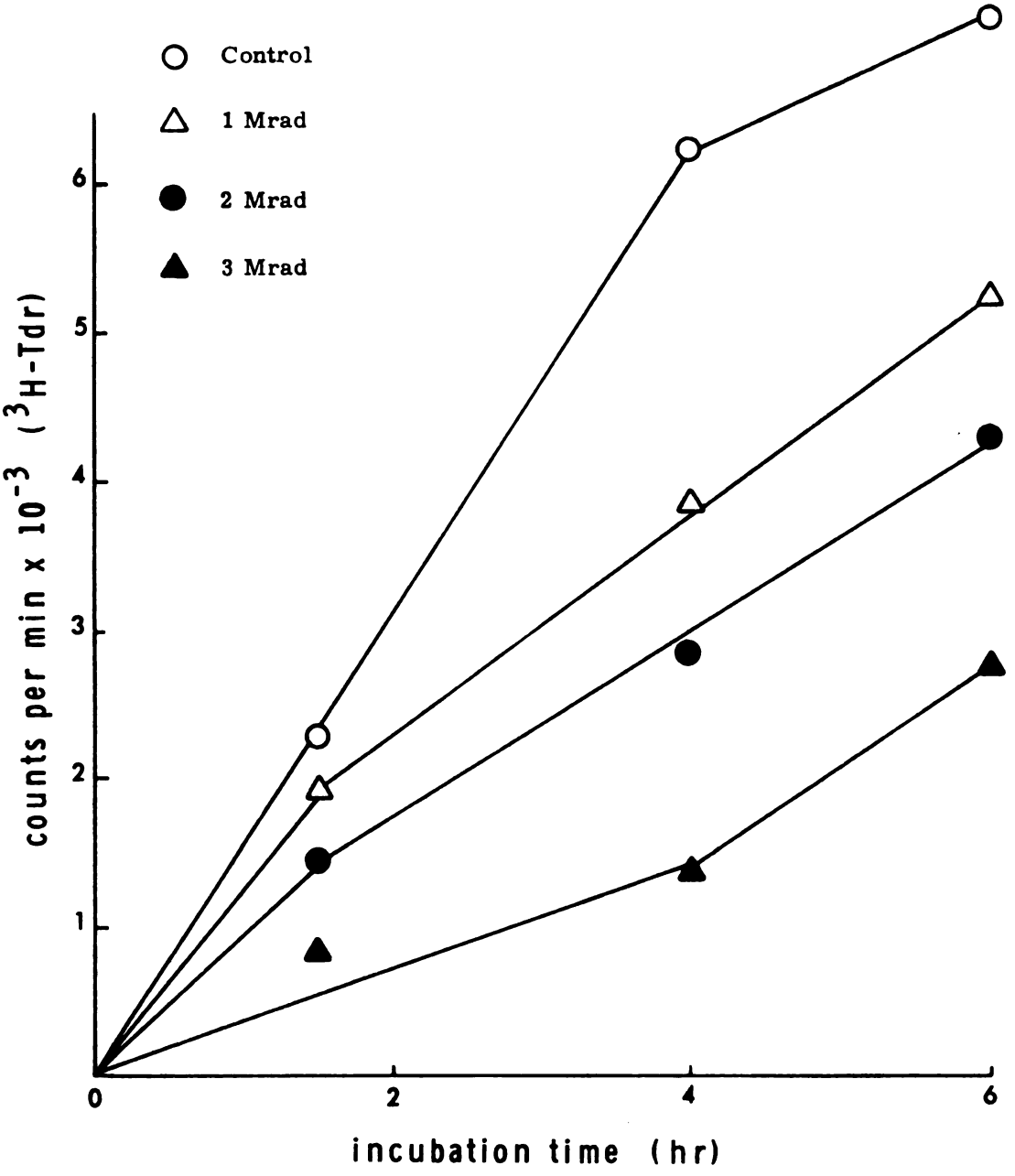

Figure 3. -- ^{3}H-thymidine uptake in DNA synthesis by AV_3 cells incubated with 5.8×10^{-2}M irradiated sucrose solutions (preliminary experiment).

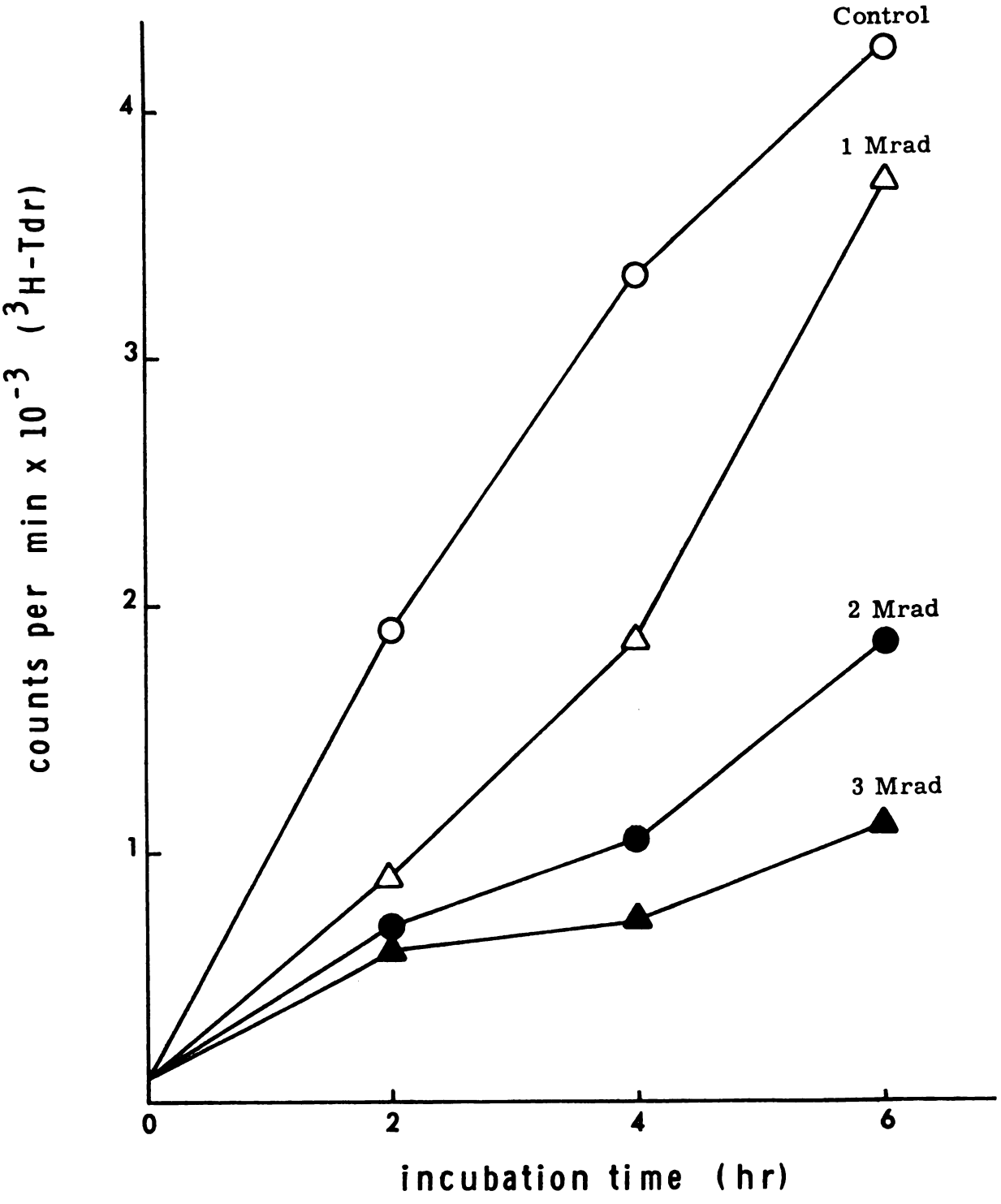

Figure 4. -- ^{3}H-thymidine uptake in DNA synthesis by AV_3 cells incubated with 2% irradiated sucrose solutions treated with activated carbon post-irradiation.

and 6). Berry, Hills and Trillwood (1965) were the first to report on the toxicity of irradiated glucose and fructose solutions to mammalian cells in vitro. For 1% solutions of glucose or fructose added to the growth medium the toxicity was maximal at 10^5 rads. They also found that the effect persists for more than 6 months.

The toxicity of irradiated fructose was recognized by Blank and Arnold as early as 1935. They observed that the irradiation of a ketose, or of a carbohydrate which hydrolizes to produce a ketose, brings about complete inhibition of the growth of Bacillus subtilis in a shorter period of irradiation than is required for any aldose in that class.

4. Effect of pH

When a 4% sucrose solution is irradiated to 3 Mrad, its pH falls from 7.0 to 3.2. If this solution is added to MEM in a 1:1 proportion, the resulting medium is 2% in the irradiated sucrose and its pH is raised by the buffering capacity of the MEM to around pH 6.40. It was observed that an unirradiated sucrose medium at pH 6.4 was as inhibitory as a 3 Mrad irradiated sucrose added to the medium when the synthesis of DNA was followed under

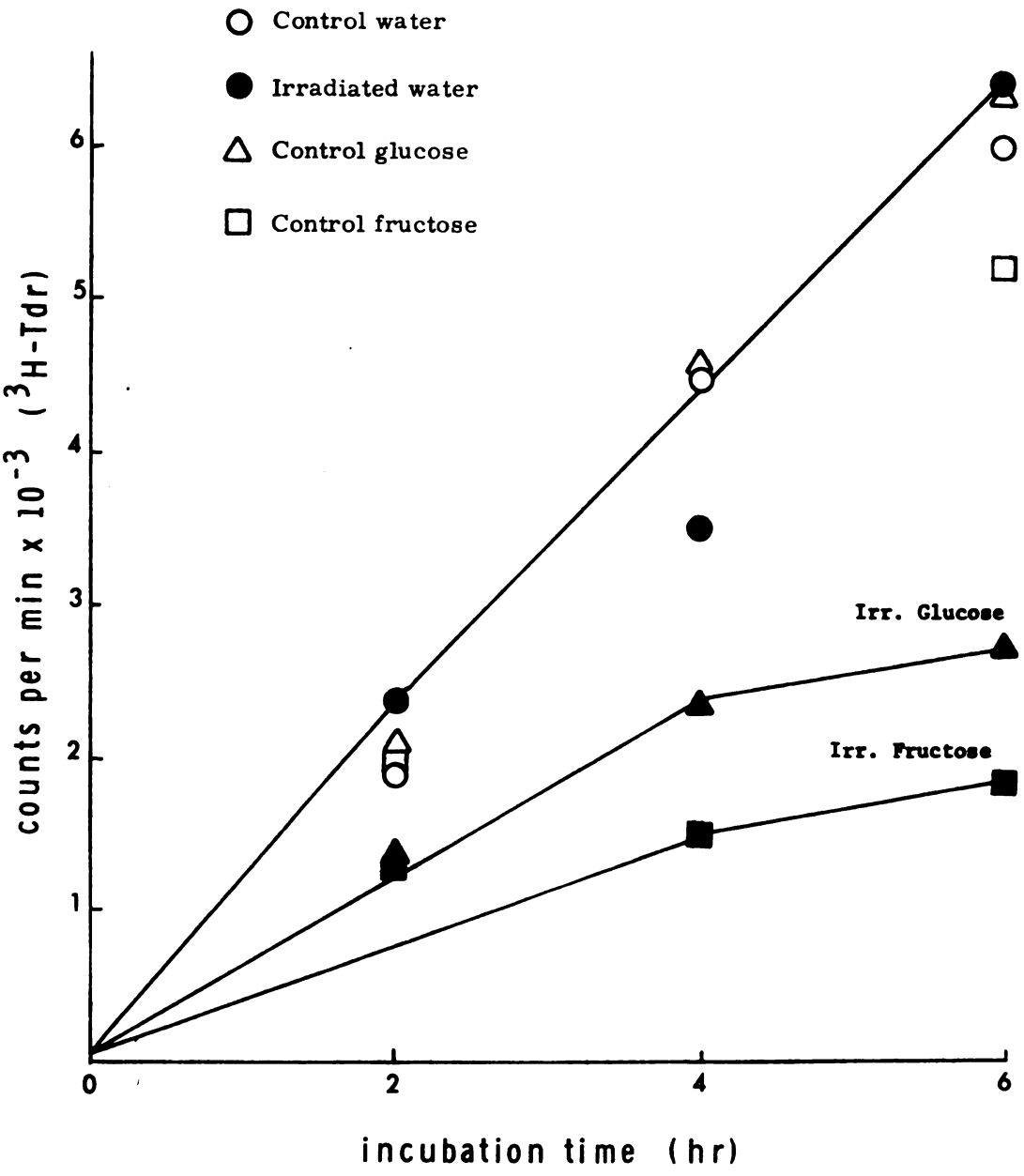

Figure 5. -- ^{3}H-thymidine uptake in DNA synthesis by AV_3 cells incubated with 5.8×10^{-2}M glucose and fructose solutions irradiated at 3 Mrad.

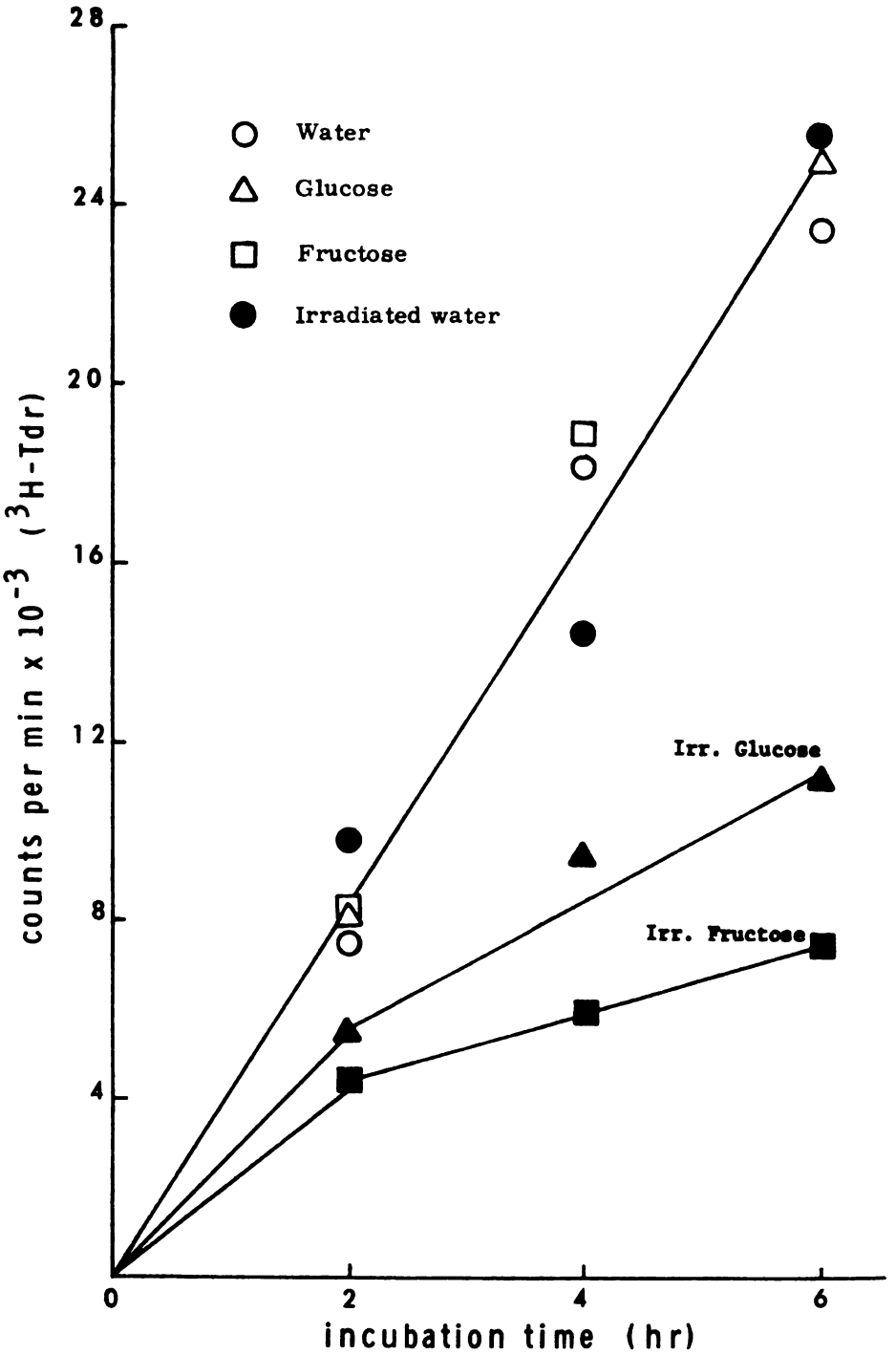

Figure 6. -- ^{3}H-thymidine uptake in DNA synthesis by AV_3 cells incubated with 5.8×10^{-2}M glucose and fructose solutions irradiated at 4 Mrad.

similar pH conditions (Figure 7). Similar observations were made by Baumgartner (1936), who found that irradiation of 1% sucrose solution resulted in a drop in pH from 7.0 to 3.5-4.0. The use of such solutions as a base for nutrient media without subsequent pH adjustment was naturally followed by inhibition of the cell normal processes.

Equimolar concentrations (5.8×10^{-2}M) of 3 Mrad irradiated glucose, fructose or sucrose contained in growth medium were used to study the synthesis of DNA in AV_3 cells. The pH of the media containing irradiated sugars and nonirradiated controls was adjusted to 7.2 prior to incubation. No change in pH was observed at the end of the test period. Most interesting were the results obtained with irradiated glucose. A highly significant reduction in the inhibition of DNA synthesis (a drop from 60% to 12%) was observed when the pH was adjusted to 7.2 (Figure 8). It seems that these cells can incorporate thymidine into their DNA molecule at a rate comparable to the control even in the presence of 3 Mrad irradiated glucose (2%) solution. Berry, Hills and Trillwood (1965) found that irradiated glucose powder reconstituted with irradiated water proved toxic to Strain L fibroblasts, but not to the He La cell-lines.

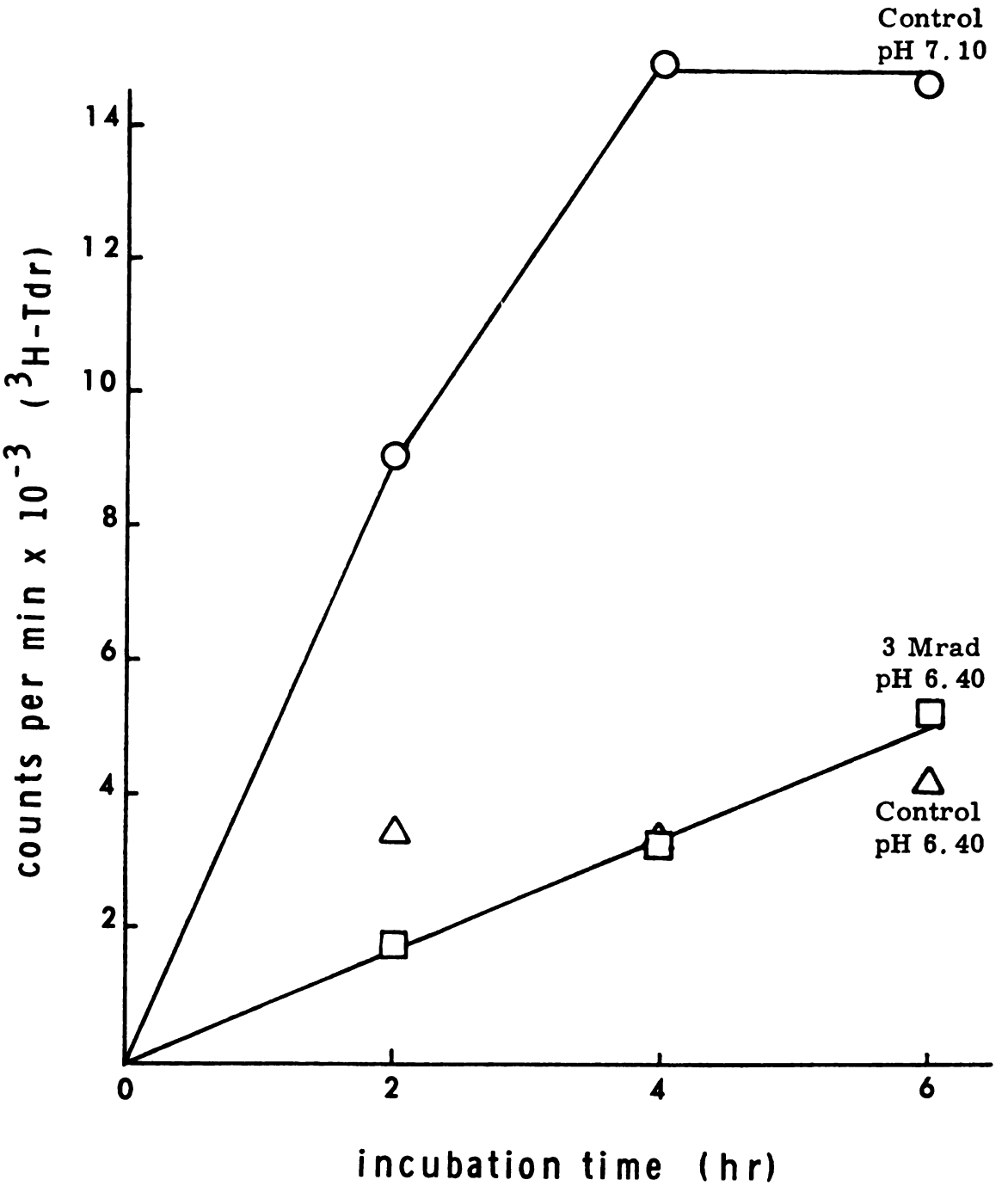

Figure 7. -- ^{3}H-thymidine uptake in DNA synthesis by AV3 cells incubated with 5.8×10^{-2}M sucrose solution irradiated at 3 Mrad and unirradiated sucrose solutions at pH 6.40 in MEM.

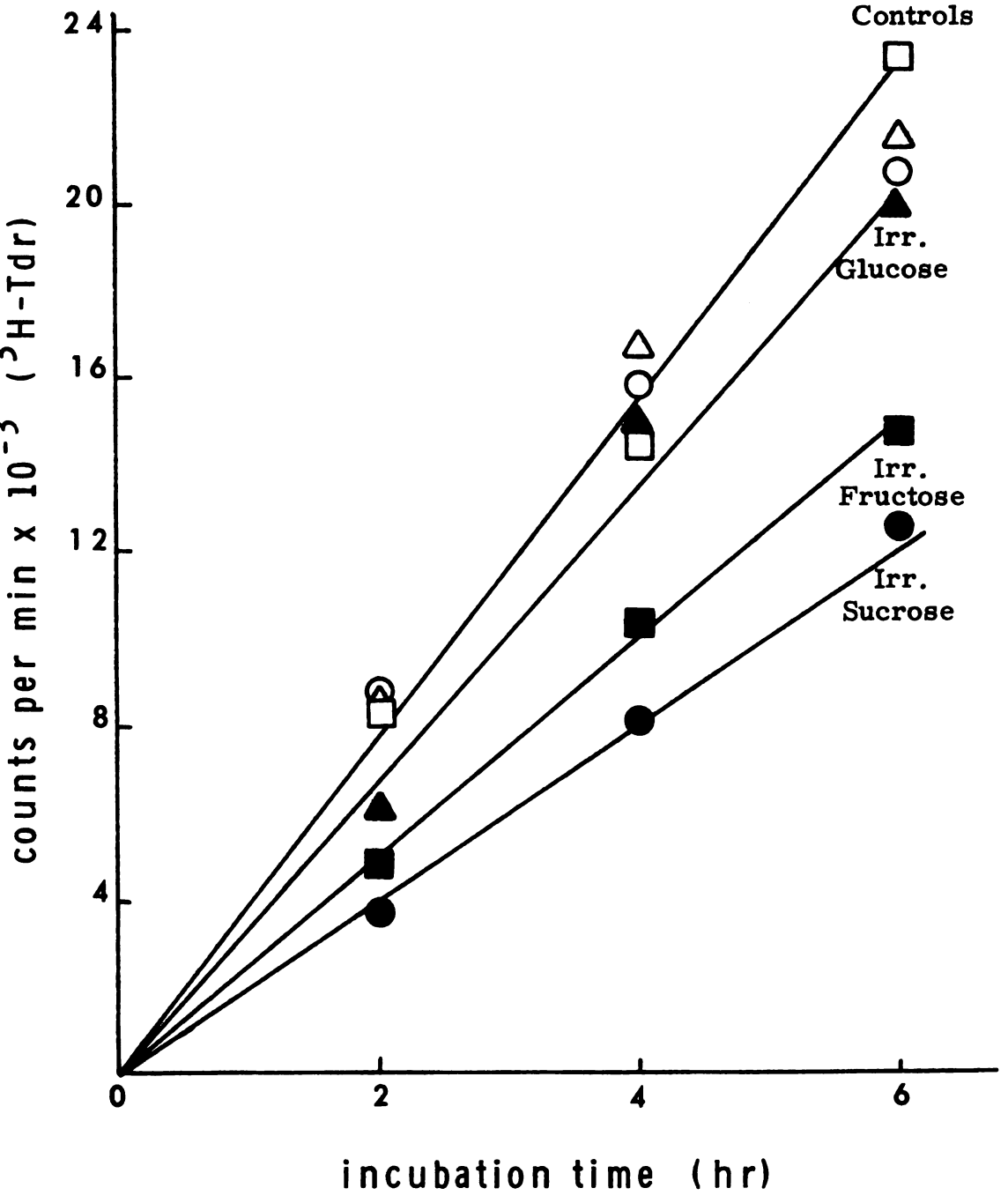

Figure 8. -- ^{3}H-thymidine uptake in DNA synthesis by AV_3 cells incubated with 3 Mrad irradiated sucrose, glucose and fructose solutions (5.8×10^{-2}M in MEM at pH 7.2).

These findings suggest that the toxic products can be handled in different ways by different cell types.

No appreciable reduction in the toxicity produced by irradiated fructose or sucrose solutions was obtained by adjusting the pH to 7.2 (Figure 8).

5. Irradiated glucose vs. DNA synthesis

The results of the experiments designed to determine the effect of irradiated glucose on the DNA synthesis of human cells are shown in Figures 8 and 9. It is clearly seen from these results that irradiated glucose does not significantly inhibit the synthesis of DNA of these cells. No difference was found between 1, 2 and 3 Mrad treatments at the end of 6 hours of incubation. The percentage of inhibition of the DNA synthesis was 33 for all 3 doses (Figure 9).

6. Irradiated fructose vs. DNA synthesis

The results shown in Figure 10 clearly demonstrate that 3 Mrad irradiated 5.8×10^{-2}M fructose induces severe inhibition (63%) of the synthesis of DNA when in contact with human cells in a rapid growth phase. A 30 to 35% inhibition was observed for the doses of 1 and 2 Mrad.

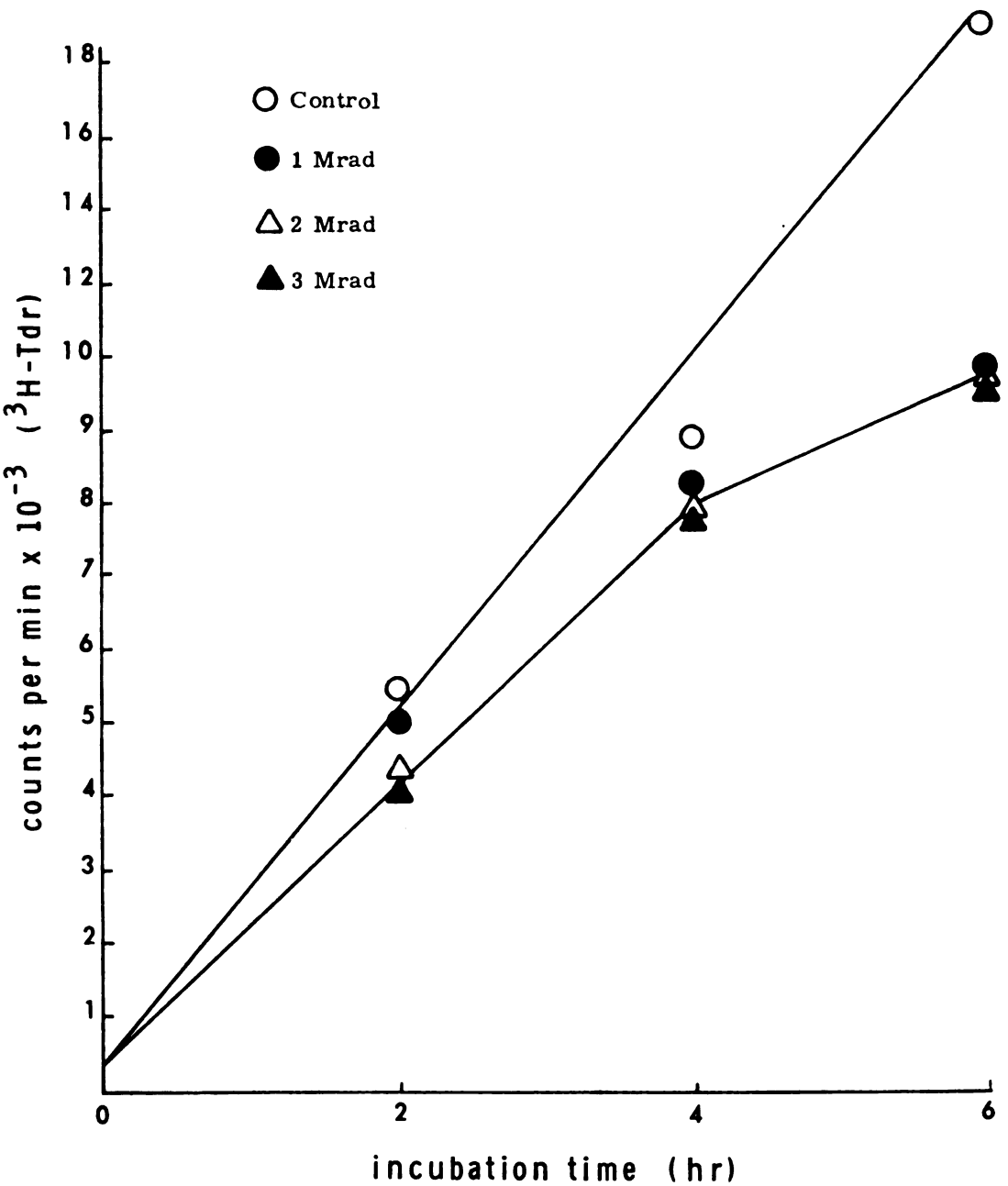

Figure 9. -- ^{3}H-thymidine uptake in DNA synthesis by AV_3 cells incubated with 1, 2 and 3 Mrad irradiated glucose solutions (5.8×10^{-2}M in MEM at pH 7.2).

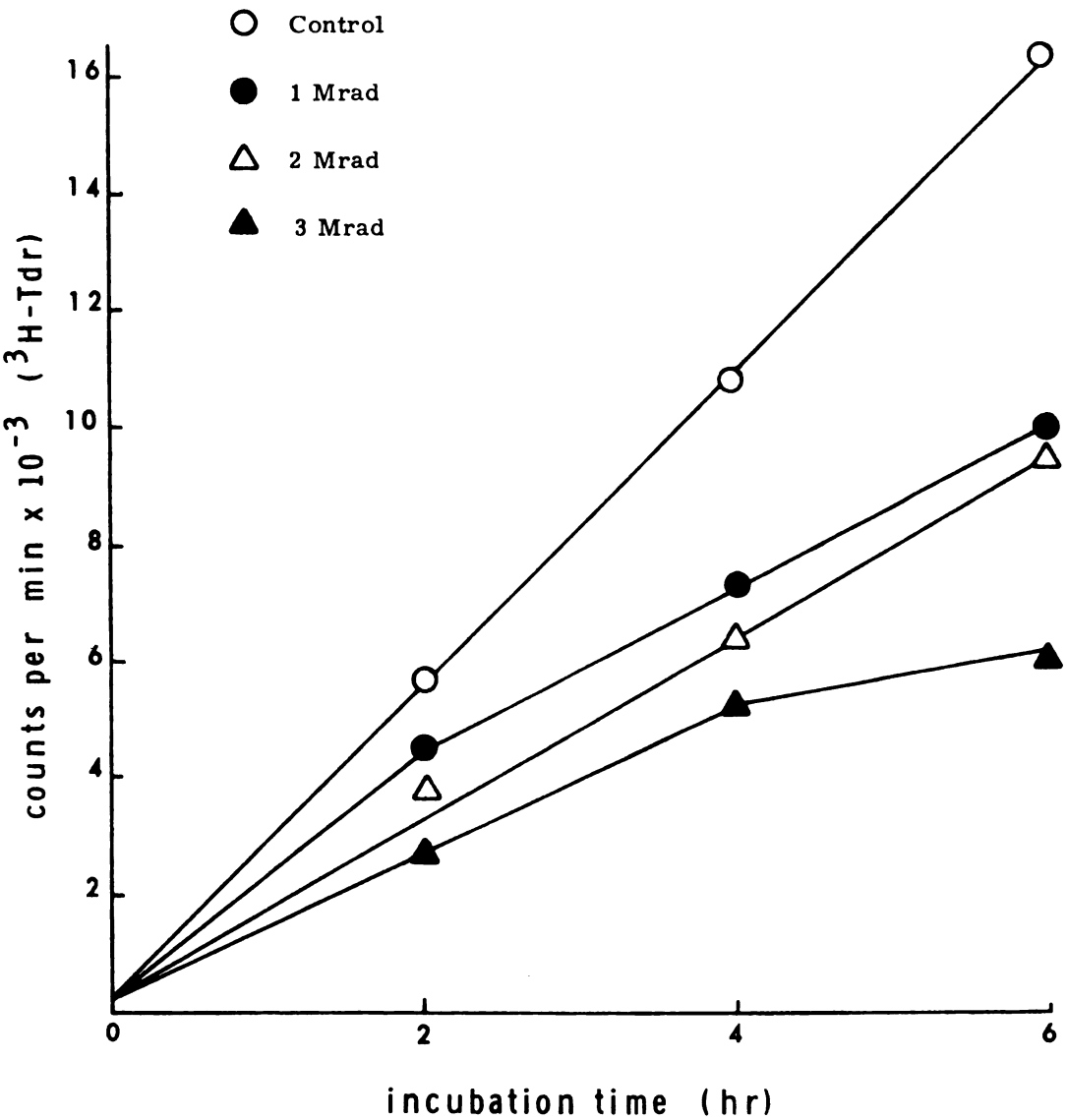

Figure 10. -- ^{3}H-thymidine uptake in DNA synthesis by AV_3 cells incubated with 1, 2 and 3 Mrad irradiated fructose solutions (5.8×10^{-2}M in MEM at pH 7.2).

7. Irradiated sucrose vs. DNA synthesis

The results of experiments conducted to determine the effect of 1, 2 and 3 Mrad irradiated 5.8×10^{-2}M sucrose solution on the incorporation of tritiated thymidine into DNA of AV_3 cells are well represented by Figure 11. The decrease in DNA synthesis is dose dependent. At the 1 Mrad dose level only 20% inhibition was observed, while 51 to 53% inhibition were obtained for the 2 and 3 Mrad irradiation doses respectively.

C. Synthesis of RNA

Since tritiated uridine is only incorporated in the molecule of RNA, it represents an excellent precursor to study the synthesis of RNA.

1. Irradiated glucose vs. RNA synthesis

Figure 12 shows the relation of the incorporation of uridine into RNA of human cells in contact with 5.8×10^{-2}M glucose irradiated at 1, 2 and 3 Mrad respectively. It was demonstrated that irradiated glucose exerts only a moderate inhibition in the synthesis of RNA of these cells. Thirteen to 15% inhibition was obtained at the 3 Mrad dose level.

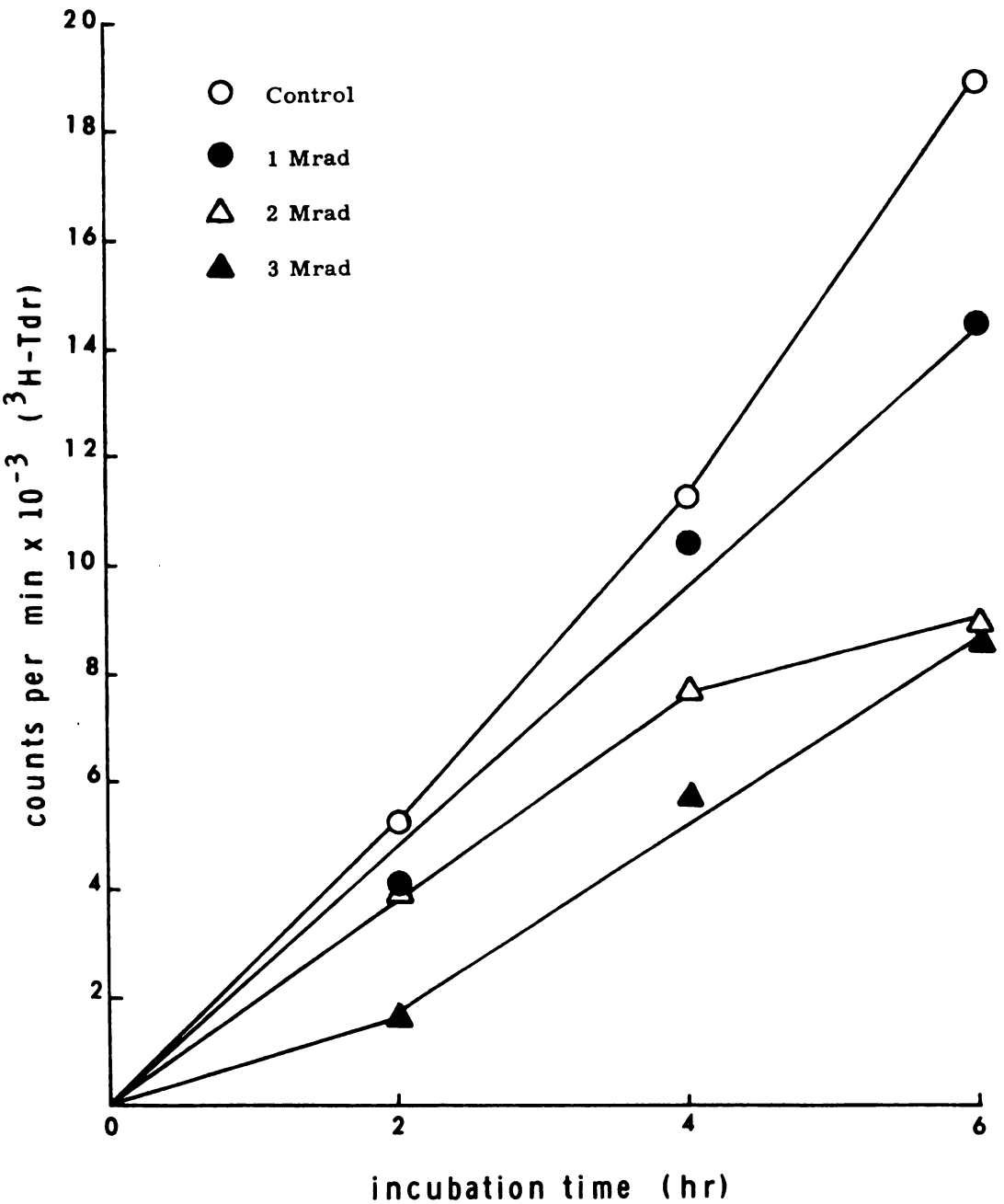

Figure 11. -- ^{3}H-thymidine uptake in DNA synthesis by AV_3 cells incubated with 1, 2 and 3 Mrad irradiated sucrose solution (5.8×10^{-2}M in MEM at pH 7.2).

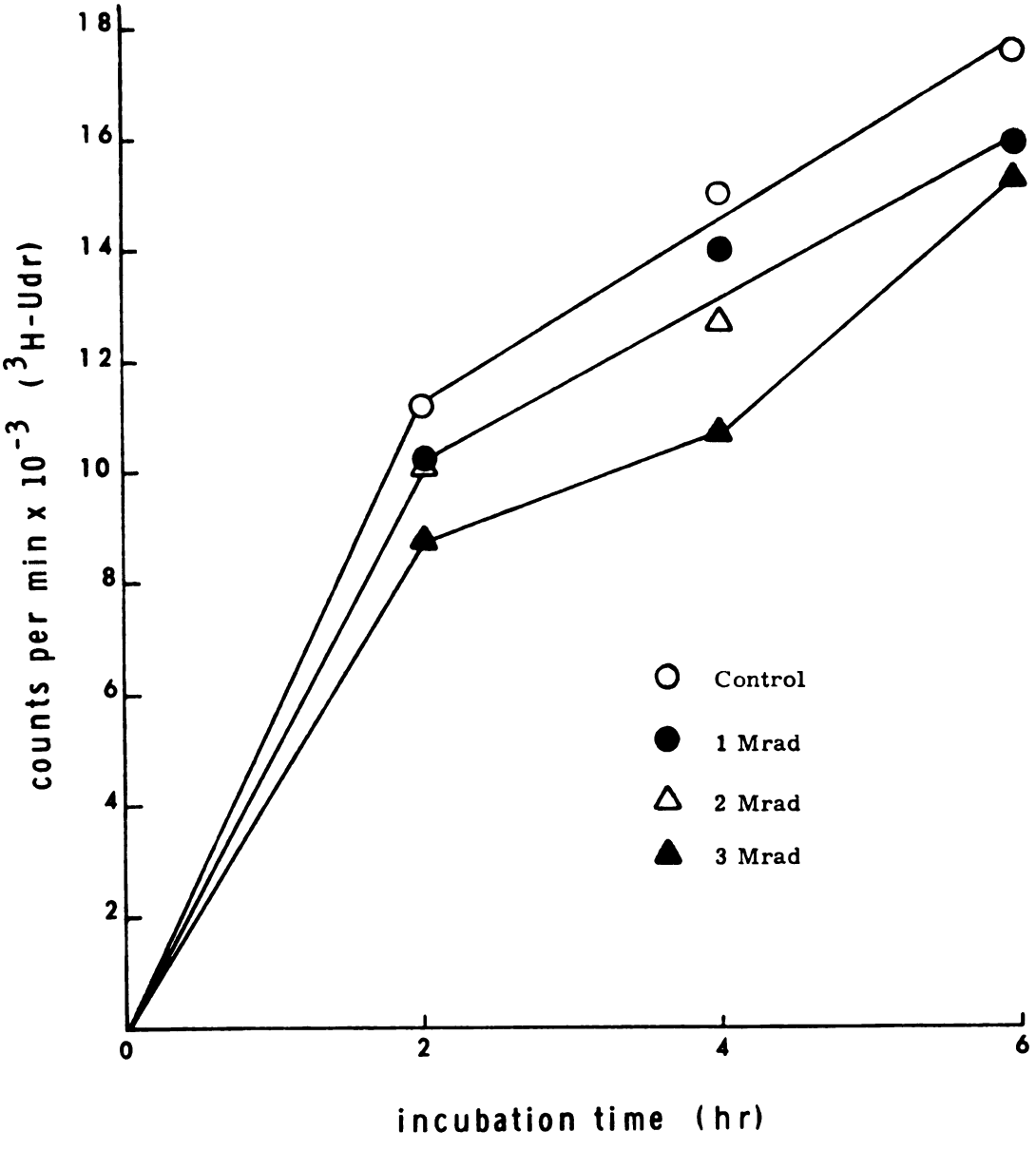

Figure 12. -- ^{3}H-uridine uptake in RNA synthesis by AV_3 cells incubated with 1, 2 and 3 Mrad irradiated glucose solutions (5.8×10^{-2}M in MEM at pH 7.2).

2. Irradiated fructose vs. RNA synthesis

It was found that under similar conditions of radiation doses, temperature, duration of exposure, pH of the medium and cell concentration, irradiated fructose reduced RNA synthesis in AV_3 cells to a degree slightly higher than that observed for irradiated glucose (Figure 13).

3. Irradiated sucrose vs. RNA synthesis

A very similar response to that obtained with fructose was apparent when irradiated sucrose solutions were added to the growth media to determine the synthesis of RNA in human cells (Figure 14).

D. Protein Synthesis

Tritiated leucine was used to follow the synthesis of protein in AV_3 cells exposed to irradiated sugars in their growth medium.

1. Irradiated glucose vs. protein synthesis

Glucose solutions irradiated at 1, 2 and 3 Mrad and added to the growth medium of AV_3 cells at a concentration of 5.8×10^{-2}M proved not to be very different from the

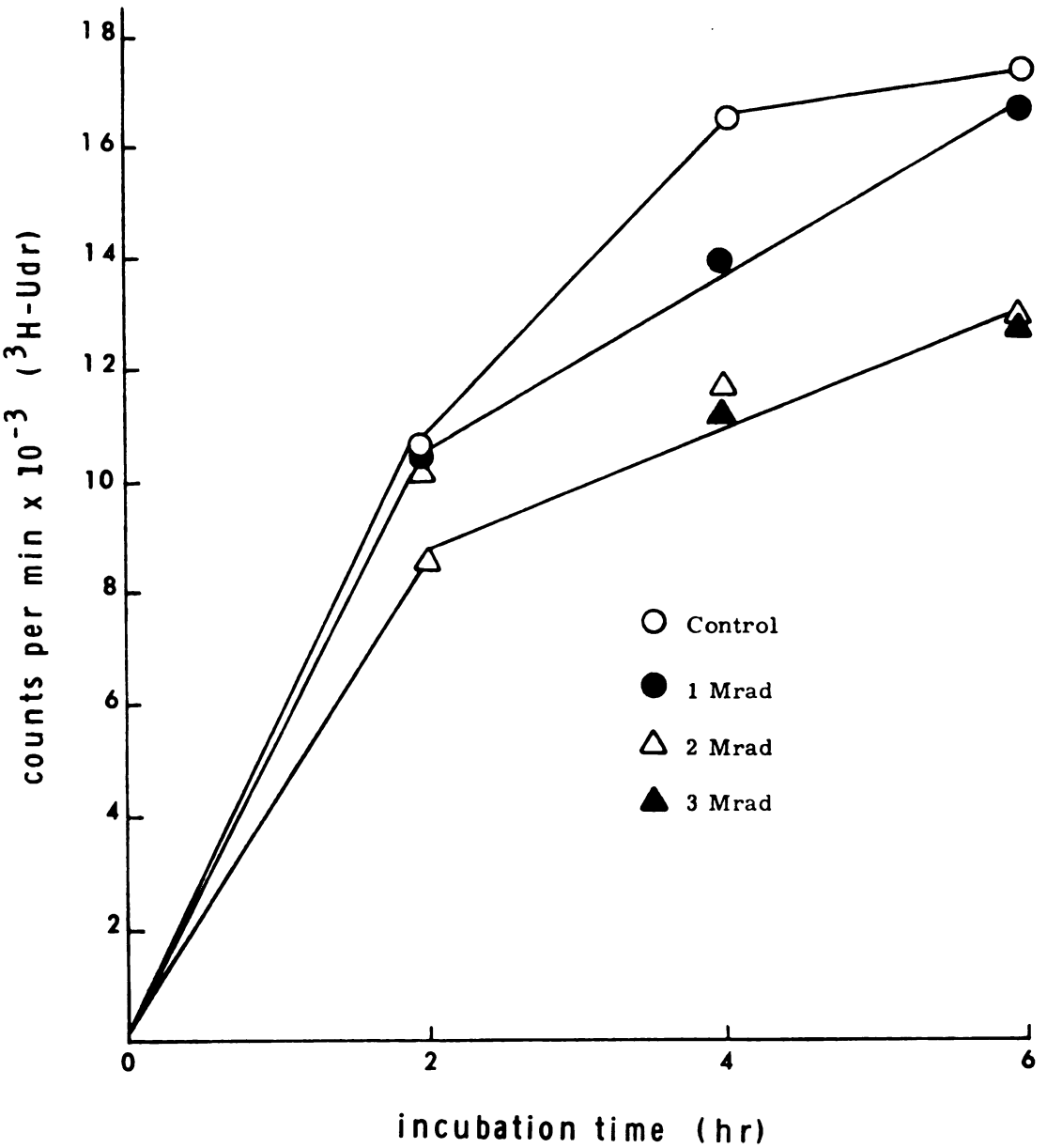

Figure 13. -- ^{3}H-uridine uptake in RNA synthesis by AV_3 cells incubated with 1, 2 and 3 Mrad irradiated fructose solutions (5.8×10^{-2}M in MEM at pH 7.2).

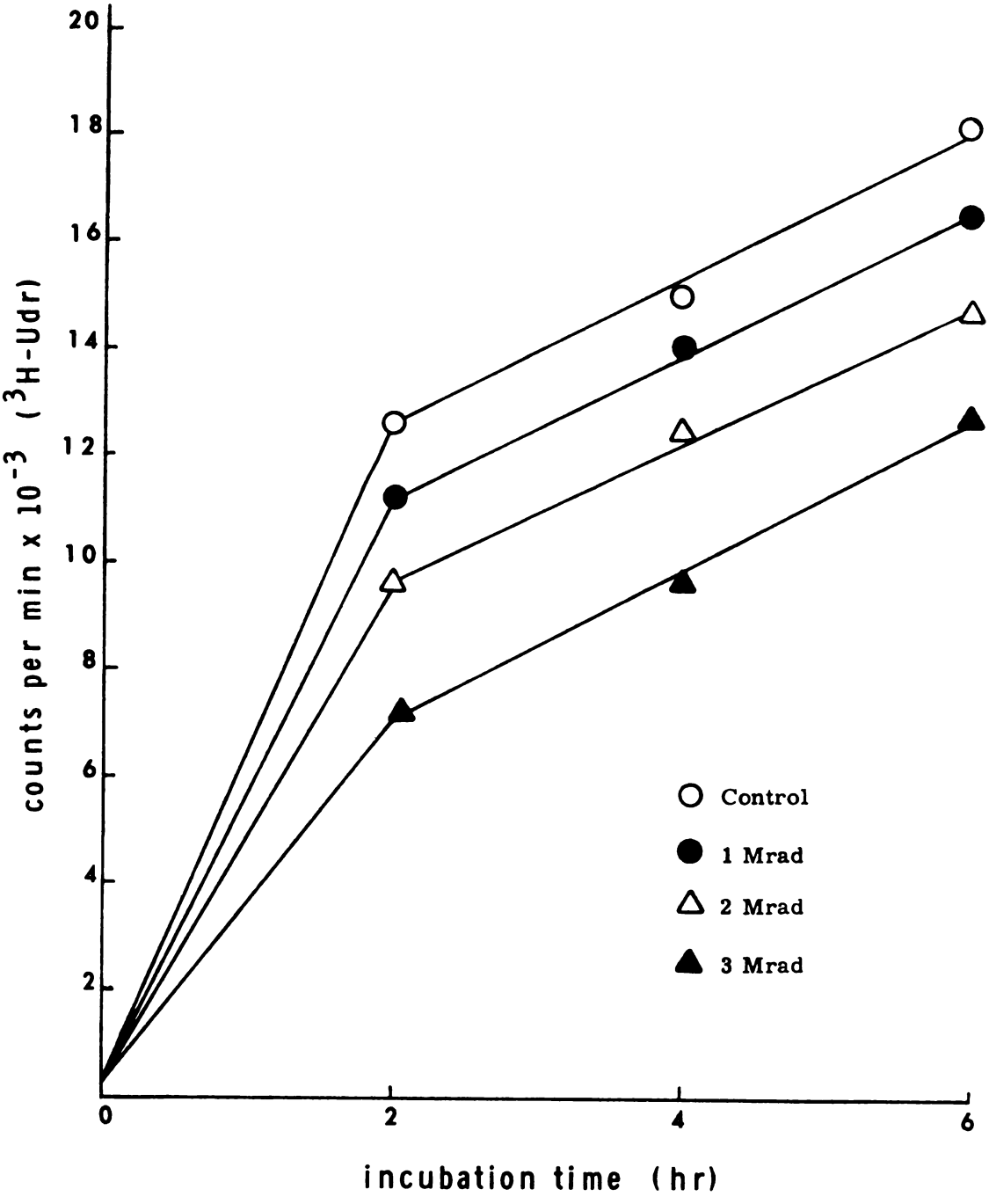

Figure 14. -- ^{3}H-uridine uptake in RNA synthesis by AV_3 cells incubated with 1, 2 and 3 Mrad irradiated sucrose solutions (5.8×10^{-2}M in MEM at pH 7.2).

unirradiated glucose when tested for toxicity related to protein synthesis. Only 20% inhibition of the protein synthesis was observed at the 3 Mrad dose level (Figure 15) after 6 hours of incubation.

2. Irradiated fructose vs. protein synthesis

The presence of irradiated fructose in the growth medium of AV_3 cells exerted a reduction on the synthesis of protein of these cells. A 41% inhibition in protein synthesis was observed at the 3 Mrad dose level (Figure 16).

3. Irradiated sucrose vs. protein synthesis

The observations from experiments performed to test the influence of irradiated sucrose on the growth medium of human cells in terms of protein synthesis are summarized in Figure 17. The inhibitory effect of irradiated sucrose on the protein synthesis of human AV_3 cells is clear at the 3 Mrad dose level. It appears that the levels of 1 and 2 Mrad are too close to a threshold effect for any definite conclusions to be derived regarding the inhibition of protein synthesis in these cells.

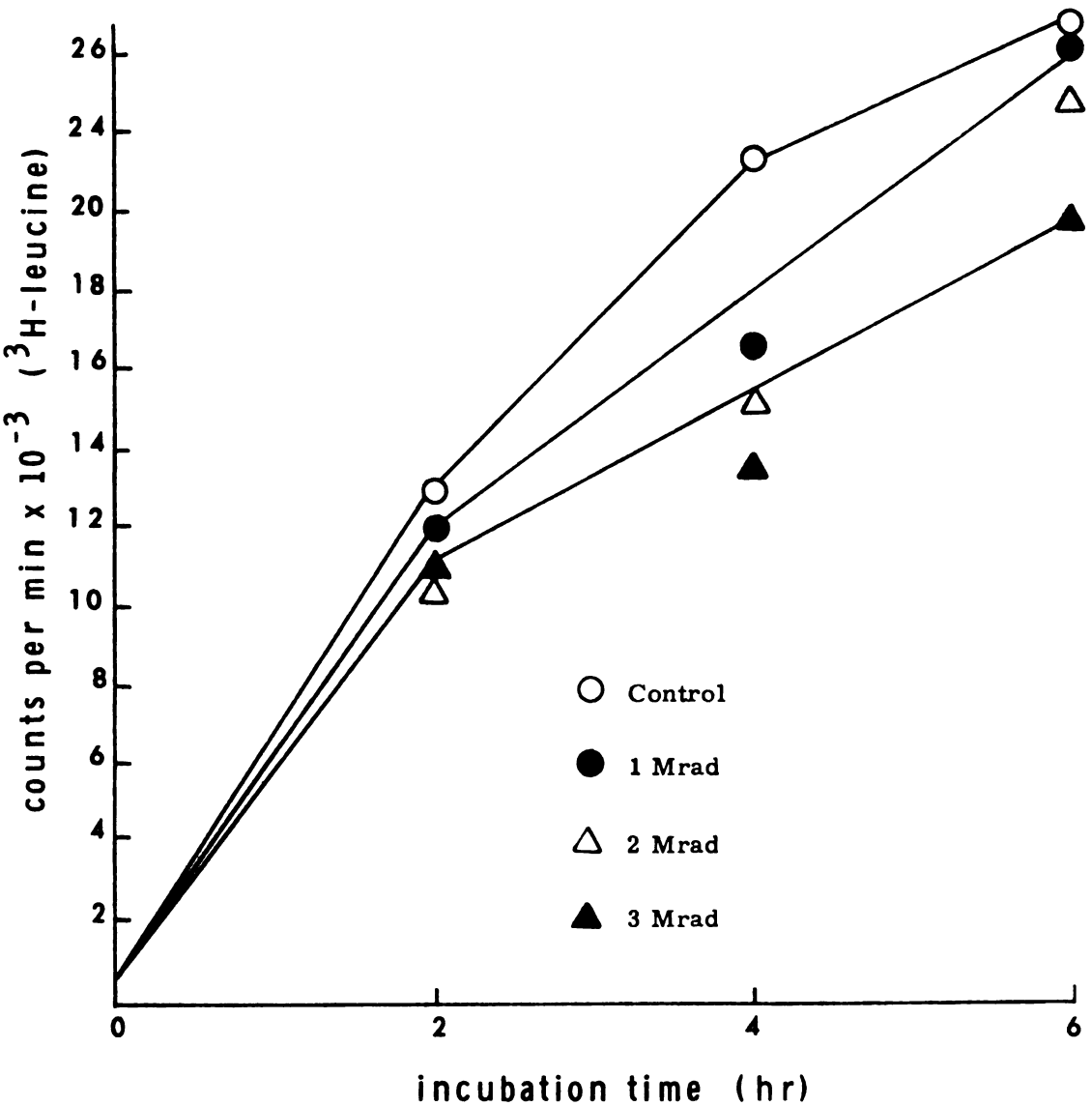

Figure 15. -- ^{3}H-leucine uptake in protein synthesis by AV_3 cells incubated with 1, 2 and 3 Mrad irradiated glucose solutions (5.8×10^{-2}M in MEM at pH 7.2).

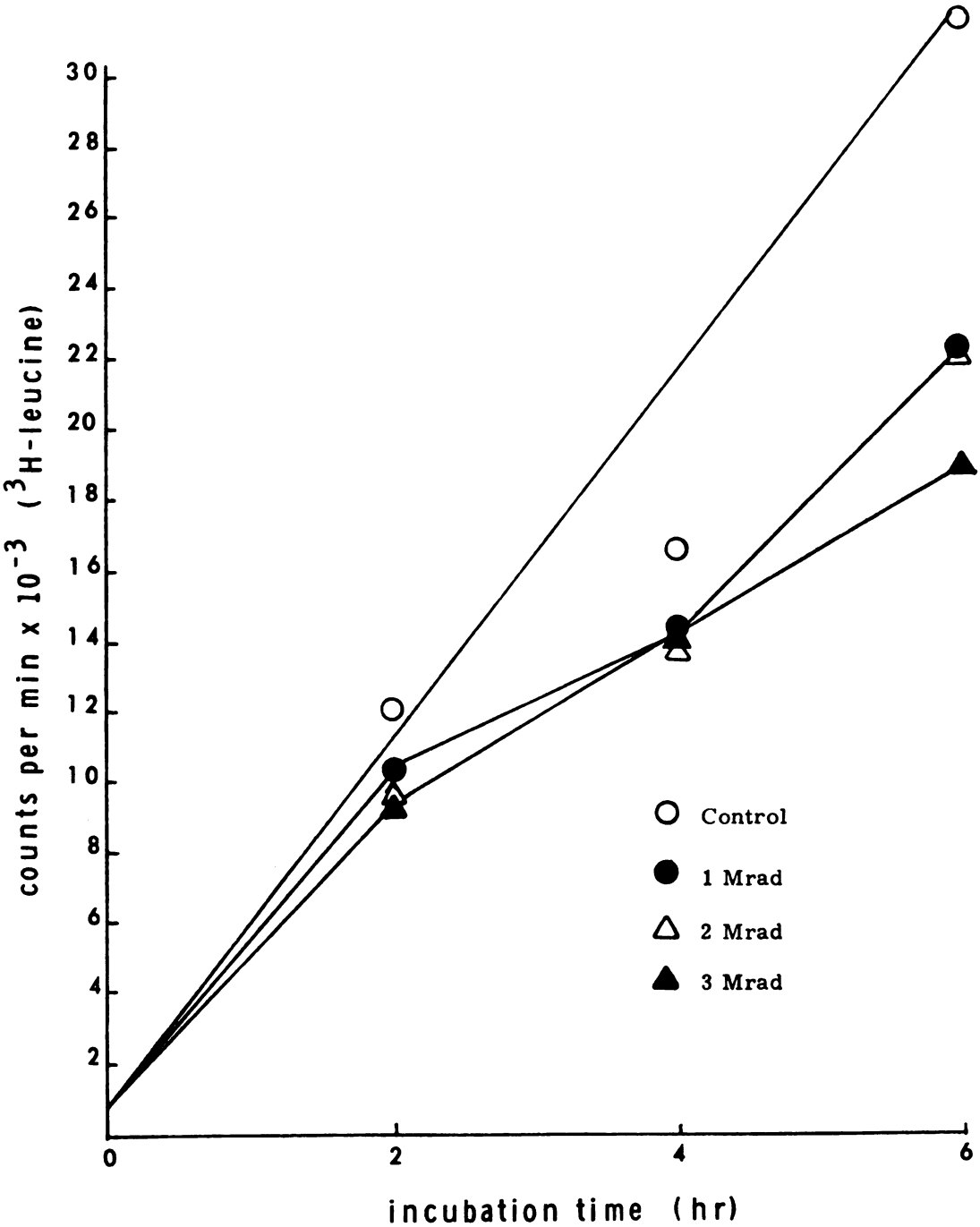

Figure 16. -- ^{3}H-leucine uptake in protein synthesis by AV_3 cells incubated with 1, 2 and 3 Mrad irradiated fructose solutions (5.8×10^{-2}M in MEM at pH 7.2).

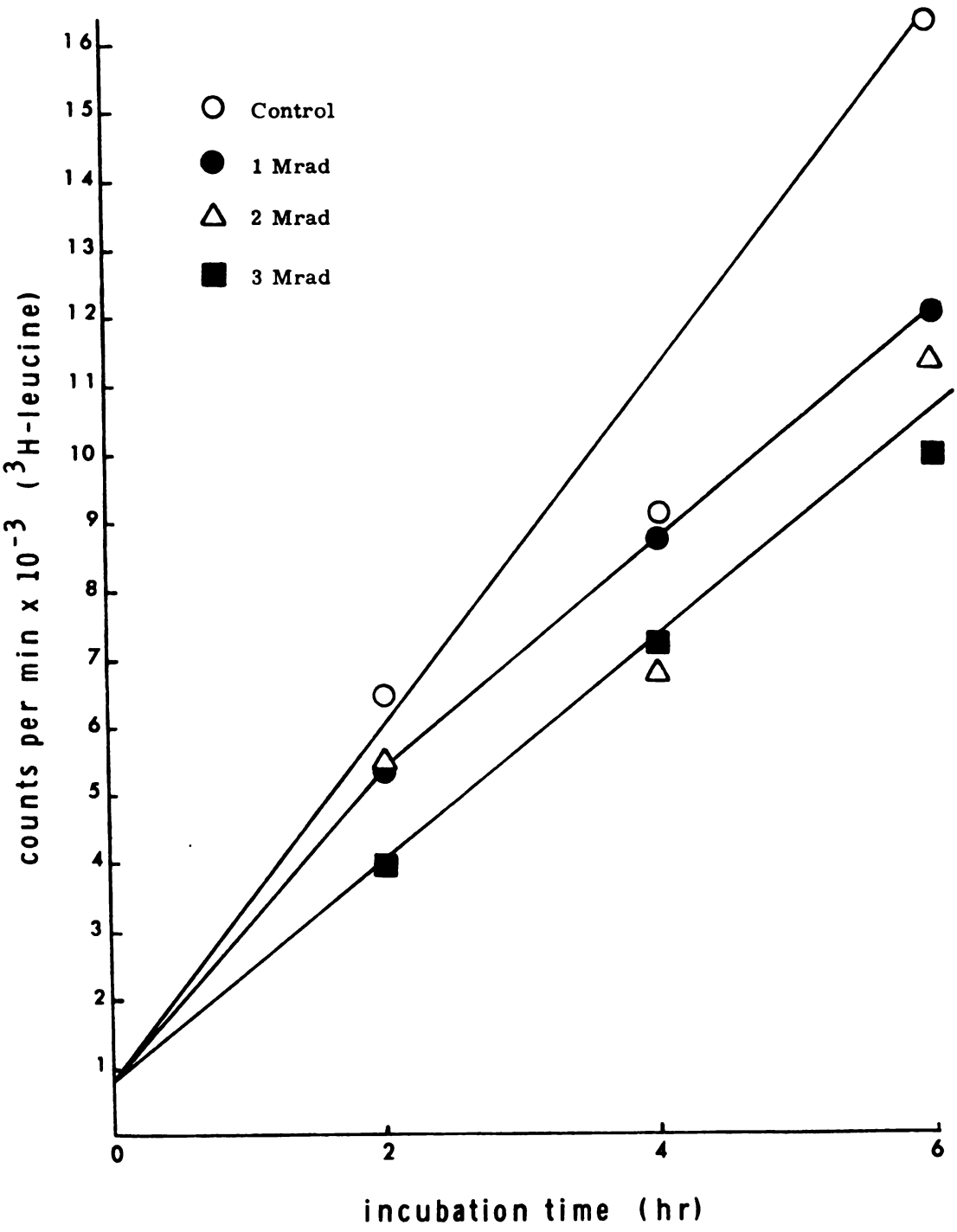

Figure 17. -- ^{3}H-leucine uptake in protein synthesis by AV_3 cells incubated with 1, 2 and 3 Mrad irradiated sucrose solutions (5.8×10^{-2}M in MEM at pH 7.2).

E. The Effect of Ageing of Irradiated Sucrose Solution on the DNA Synthesis

The ageing or storing of irradiated sucrose (3 and 4 Mrad) did not minimize its capacity to induce inhibition of the synthesis of DNA in AV_3 cells (Table 1, Figures 18 to 22). These results are in agreement with Schubert, 1967; Steward, 1967; Molin and Ehrenberg, 1964; Berry, Hills and Trillwood, 1965; and Holsten et al., 1965, who reported inhibitory effects of irradiated sugars (on bacterial and plant growth) after storage for long periods at low temperature and low pH.

Table 1. --Percentage of DNA synthesis inhibition in AV_3 cells observed using irradiated sucrose stored from 0 to 7 months at room temperature.

Dose	% Inhibition			
	0 Months	2 Months	4 Months	7 Months
3 Mrad	52	60	46	75
4 Mrad	68	75	53	87

F. DNA Polymerase Activity

Since the DNA synthesis was shown to be impaired by the presence of irradiated sucrose in the growth medium, an

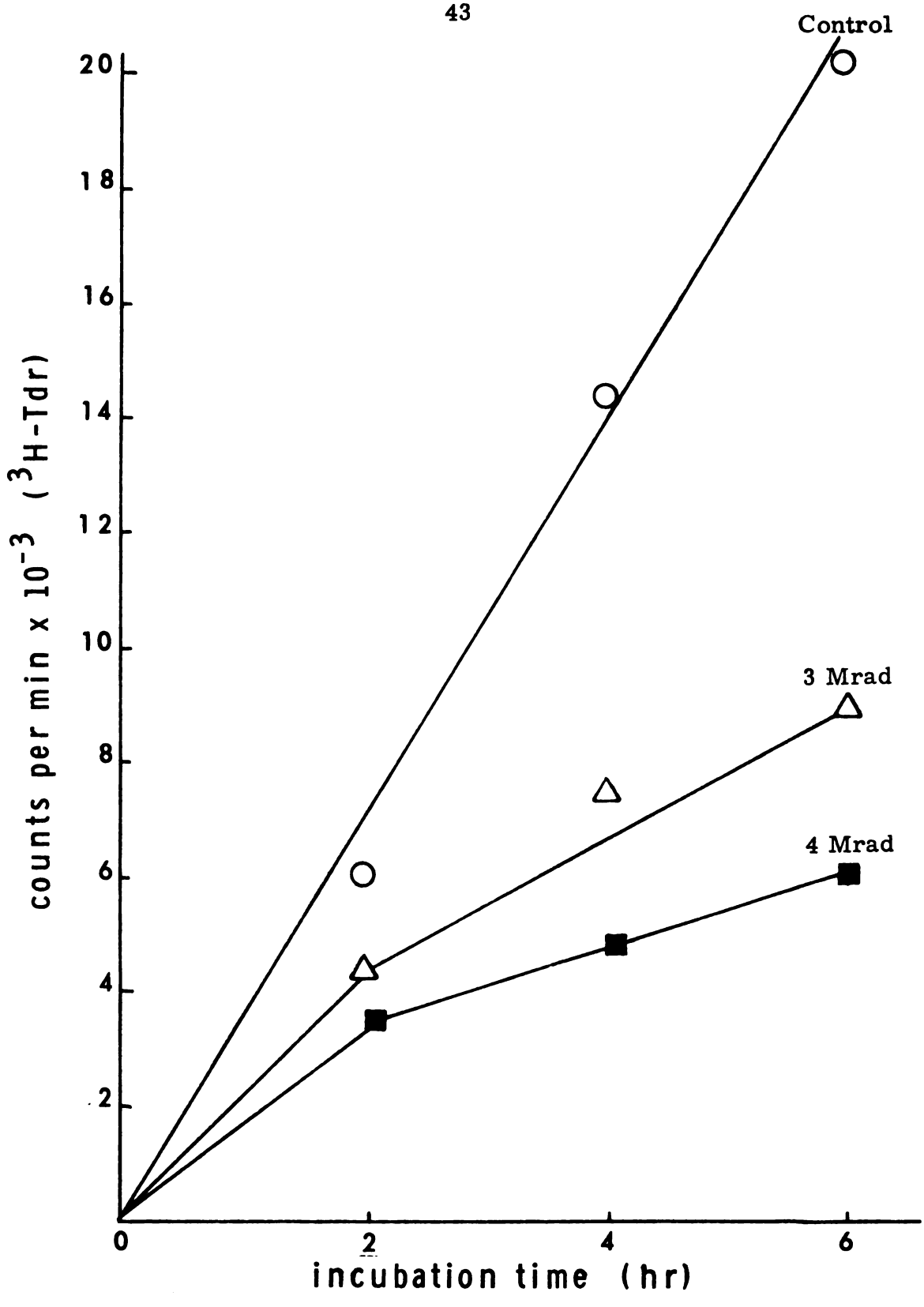

Figure 18. -- ^{3}H-thymidine uptake in DNA synthesis by AV_3 cells incubated with 3 and 4 Mrad irradiated sucrose solutions (5.8×10^{-2}M) immediately after irradiation.

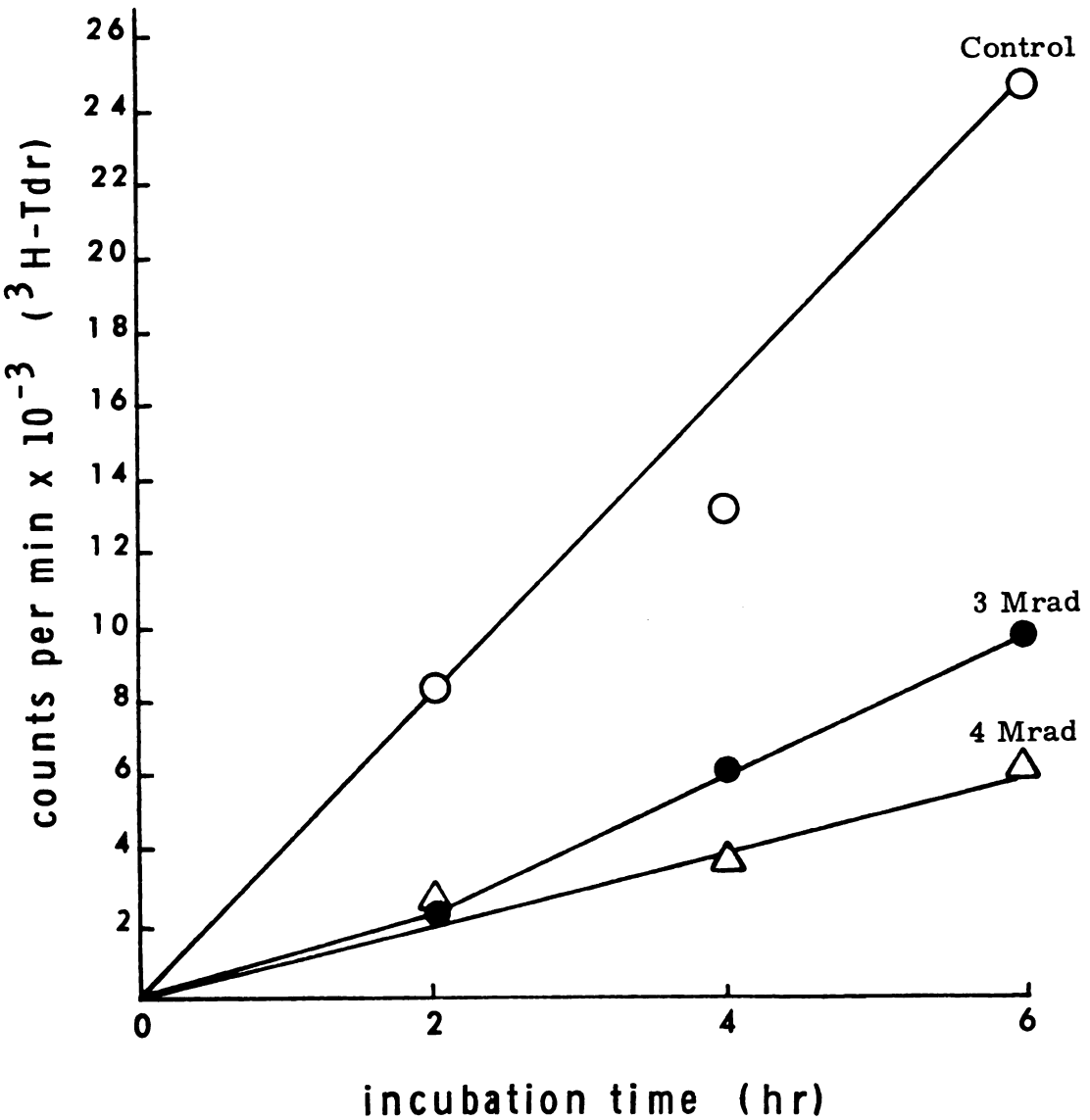

Figure 19. -- ^{3}H-thymidine uptake in DNA synthesis by AV_3 cells incubated with 3 and 4 Mrad irradiated sucrose stored for 2 months at room temperature. Sucrose solutions (5.8×10^{-2}M).

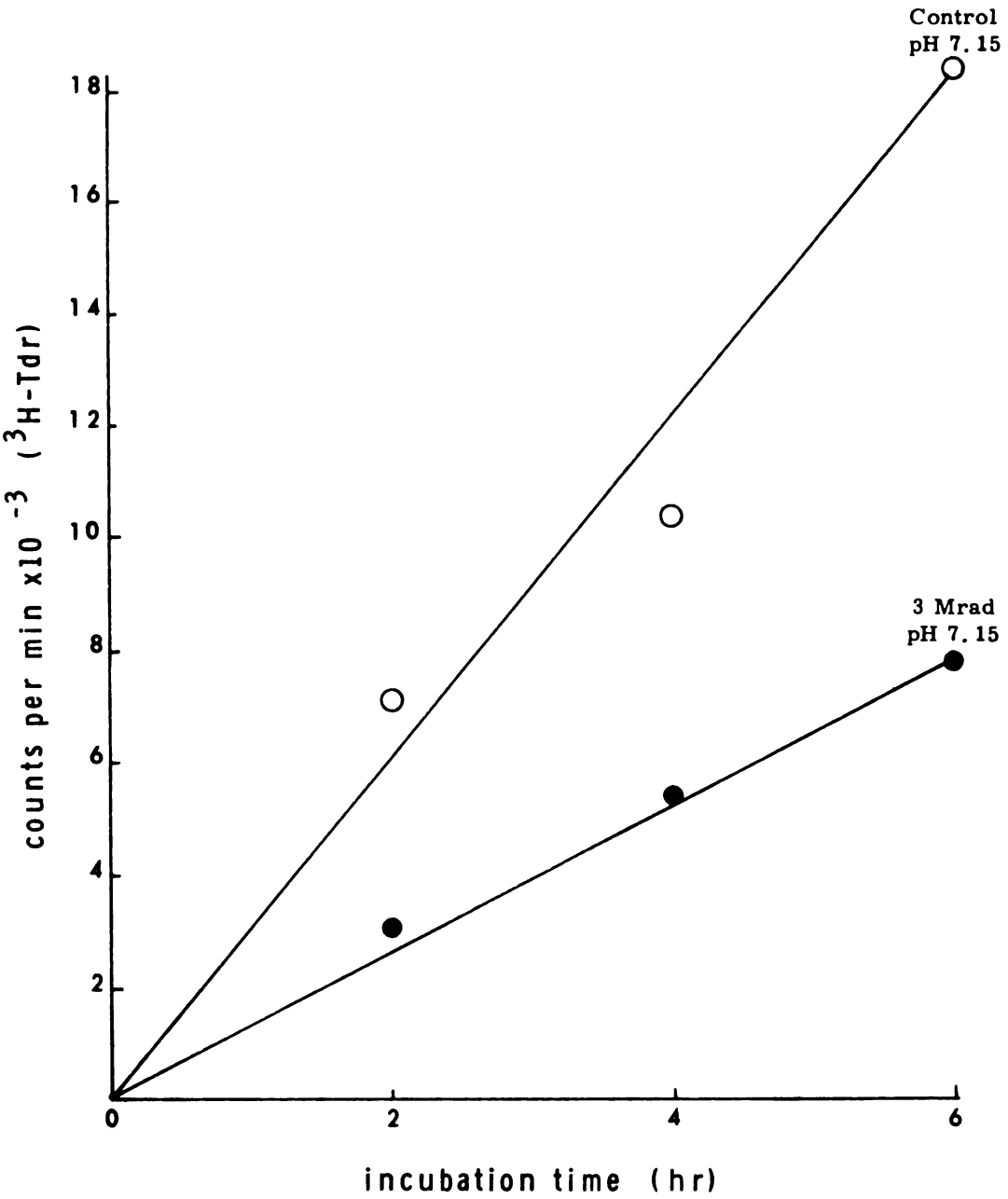

Figure 20. -- ^{3}H-thymidine uptake in DNA synthesis by AV_3 cells incubated with 3 Mrad irradiated sucrose solution stored for 2 months at room temperature. Sucrose concentration was $5.8 \times 10^{-2}M$ in MEM at pH 7.15.

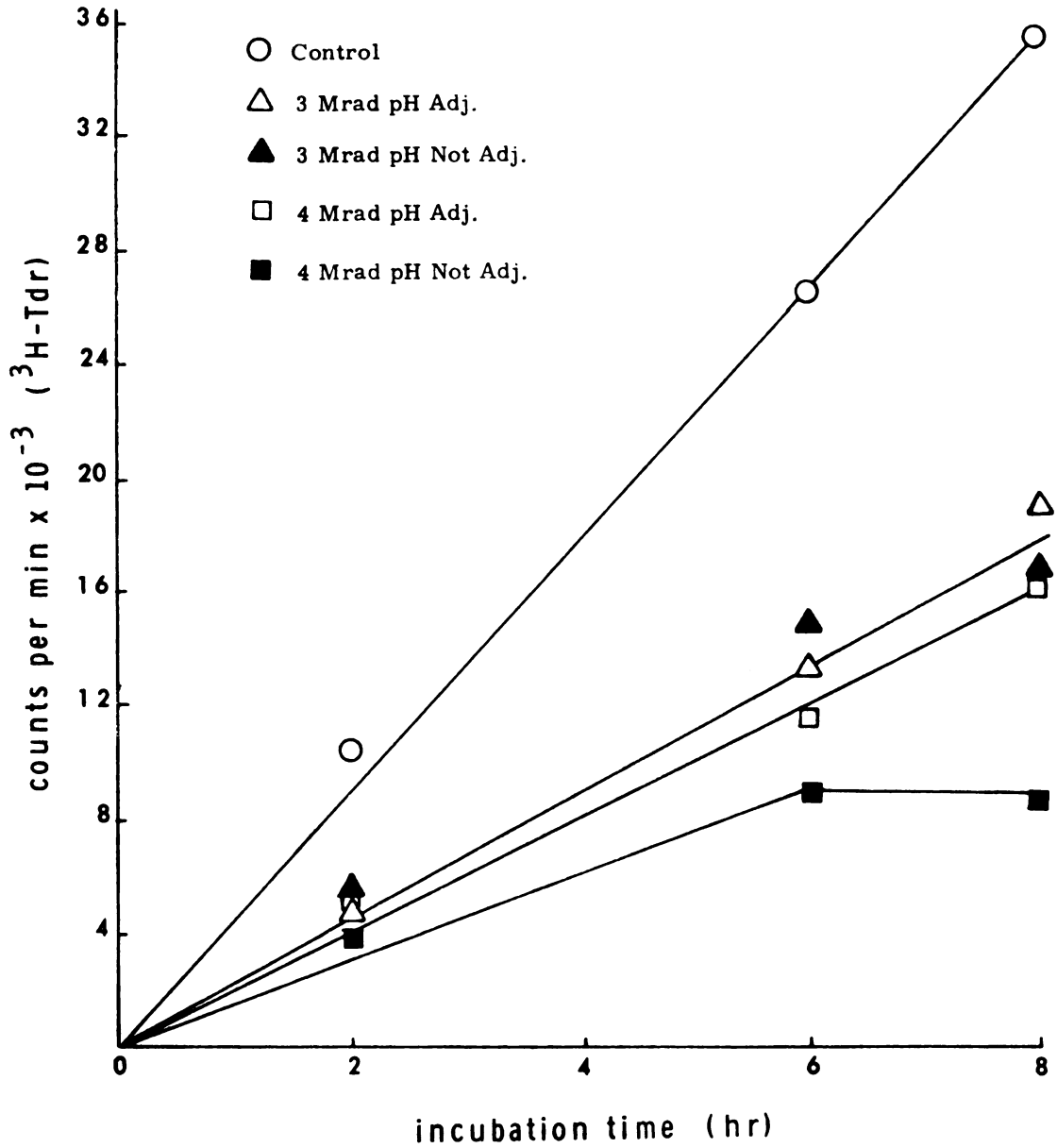

Figure 21. -- ^{3}H-thymidine uptake in DNA synthesis by AV_3 cells incubated with 3 and 4 Mrad irradiated sucrose stored for 4 months at room temperature. Sucrose concentration was 5.8×10^{-2}M in MEM at various pH's.

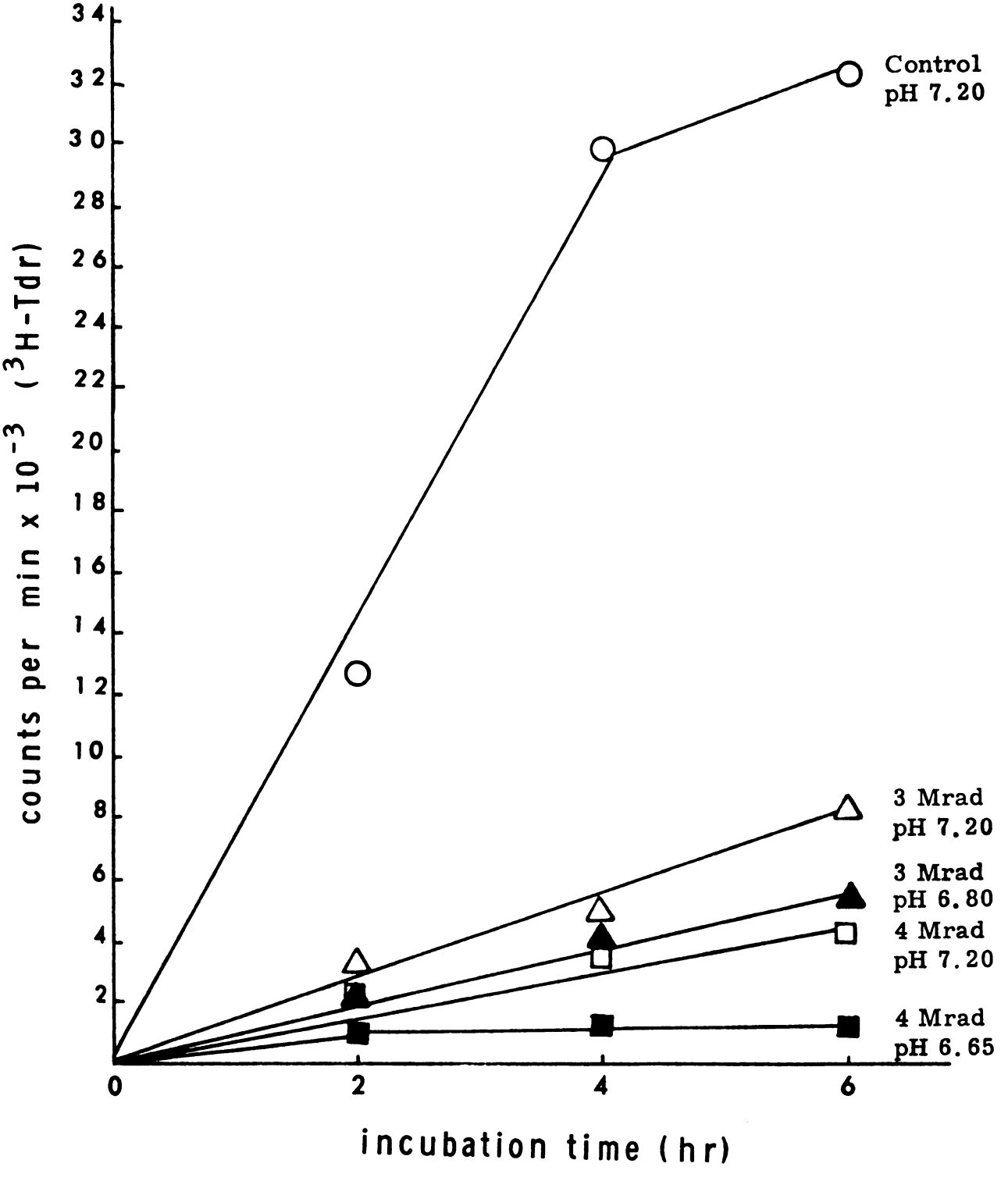

Figure 22. -- ^{3}H-thymidine uptake in DNA synthesis by AV_3 cells incubated with 3 and 4 Mrad irradiated sucrose stored for 7 months at room temperature. Sucrose concentration was 5.8×10^{-2}M in MEM at various pH's.

experiment was conducted to test whether the action of DNA polymerase is affected by the irradiated (3 Mrad) sucrose. The assay for the activity of DNA polymerase was performed using the method described by Zimmerman (1966). The results are presented in Table 2.

No significant difference in activity of the DNA polymerase was observed when 3 Mrad irradiated sucrose was present in the substrate. The inhibition of the synthesis of DNA in AV_3 cells might be caused by an impairment in the production of its precursors.

G. The Influence of Catalase on the Medium Containing Irradiated Sucrose

Molin and Ehrenberg (1965) found that the antibacterial effects of irradiated glucose was abolished by adding catalase to the irradiated solution 60 minutes prior to inoculation. Schubert (1969) provided evidence of the cytotoxicity of irradiated sucrose solutions for Salmonella typhimurium. He reported that the inhibitory action of sucrose is attributed to organic peroxides. The addition of catalase prior to or shortly after inoculation of the organism into the growth medium containing irradiated sucrose eliminated most of the inhibitory

Table 2. --DNA polymerase activity in extracts of AV_3 cells incubated with 3 Mrad irradiated sucrose for 1 hour at 37° C.[a, b]

Tube Number	H_2O	3 Mrad Sucrose	Control Sucrose	Counts per Minute
1	85	--	--	2,077
2	85	--	--	
3	--	--	85	2,201
4	--	--	85	
5	65	20	--	2,331
6	65	20	--	
7	65	--	20	2,111
8	65	--	20	
9	45	40	--	2,191
10	45	40	--	
11	45	--	40	2,274
12	45	--	40	
13	85	--	--	2,168
14	85	--	--	
15	5	80	--	1,878
16	5	80	--	
17	5	--	80	2,187
18	5	--	80	
19	85	--	--	2,224
20	85	--	--	
21	--	--	85	2,199
22	--	--	85	

[a]Each tube contained 25λ Tris buffer 1M pH 7.6; 5λ 0.1M $MgCl_2$; 10λ 40mM Mercapto ethanol; 50λ denatured DNA primer; 50λ ^3H-dNTP and 25λ extract from AV_3 cells. The total volume per tube was 250λ.

[b]Activity measured as counts/min of incorporated labeled precursors into DNA.

action produced by the irradiated sucrose solutions. Berry, Hill and Trillwood (1965) added catalase to a growth medium for mouse Strain L fibroblasts containing irradiated glucose. Incubation with catalase (5 mg/ml) for 30 to 60 minutes prior to the addition of cells to the media failed to protect against toxic effects of irradiated glucose.

Two experiments were performed to determine whether catalase could protect AV_3 cells against the deleterious effects already observed when these cells were in contact with 3 Mrad irradiated sucrose.

A beef liver catalase preparation (Sigma Chemical Company, Inc., St. Louis) containing 33,000 Sigma units/ml, 20μg/ml (1 unit decomposes 1 millimole H_2O_2 per minute at pH 7.0 at 25° C) was used for the experiment. This preparation was diluted to a final concentration of 20μg/ml. The incubation for the different treatments was done at 25° C. At the end of the incubation periods (from 20 minutes to 3 hours), MEM containing tritiated thymidine was mixed in 1:1 proportion and the synthesis of DNA was followed for 4 hours. The values in Table 3 show the averages of duplicate counts for each experiment. The presence of catalase in as much as 40 μg/ml for 3 hours of pre-incubation only partly (50 to 67% of control)

eliminated the inhibitory effect of 3-Mrad-irradiated sucrose on the synthesis of DNA in human amnion cells. It seems that peroxides are not the only toxic radiolytic product of irradiated sucrose for these cells.

Table 3. --The effect of catalase on 3-Mrad-irradiated sucrose, measured by the incorporation (in counts/min) of tritiated thymidine into the DNA of AV_3 cells.[a]

Treatment	Counts per Minute
Experiment 1 Catalase = 20 μg/ml	
Control + catalase	12,744
Heat inactivated catalase	5,835
20 minute incubation + catalase	5,435
3 hour incubation + catalase	6,487
Experiment 2 Catalase = 40 μg/ml	
No catalase, no irradiation	17,985
No catalase + irradiation	8,057
Catalase, no irradiation	15,105
Heat inactivated catalase	10,250
20 minute incubation + catalase	9,146
3 hour incubation + catalase	11,779

[a]Counts per minute at 4 hours after inoculation of the media.

H. Reducing Sugar Content of Irradiated Sucrose

The amount of reducing sugars obtained from irradiated sucrose has been reported by several investigators (Phillips, 1960, 1963; Steward et al., 1967; Schubert, 1969). The figures vary depending on the physical state of the sugar, the temperature, pH and ultimately the radiation dose.

It was considered important to know how much total reducing sugars (glucose and fructose) are produced by the radiation doses in this study. The determination of reducing sugars was performed by the colorimetric method using 3, 5 dinitrosalicylate reagent as indicated by Clark (1964). The results shown in Table 4 are expressed as percent total reducing sugar. The values are in agreement with the findings of other investigators (Schubert, 1969; Steward et al., 1967). The analyses were performed 5 days after the irradiation of the sucrose solution.

Table 4. --Percent of total reducing sugars from 1, 2 and 3 Mrad irradiated sucrose solution.

Dose	% Reducing Sugars	Average
1 Mrad	2.35 2.76	2.56
2 Mrad	5.00 5.34	5.17
3 Mrad	6.32 7.56	6.94

Table 5 summarizes the results obtained from all the experiments performed in this study in which AV_3 cells were incubated with 3 Mrad irradiated sugars for 6 hours.

Table 5. --Average percent inhibition of DNA, RNA and protein synthesis for AV_3 cells in contact with 3 Mrad irradiated sugars. (Duration of experiment = 6 hours)

	DNA	RNA	Protein
Sucrose	49	26	38
Glucose	20	14	24
Fructose	51	26	34

Discussion

The interpretation of results obtained from the action of irradiated sugars on biological systems is a rather difficult task due to the complexity of the reactions involved. It is well established that irradiated sugar solutions are toxic to bacteria, plants and mammalian cells in culture.

The results of the studies performed using AV_3 cells as test subjects for the action of irradiated sugars are in agreement with the behavior of most of the biological material tested previously. The inhibitory effects on growth and reproduction of the cells were more pronounced when the time the cells were in contact with the

irradiated sugar was longer. The removal of the toxic medium and the addition of fresh normal growth medium did not improve the condition of the already damaged cells. Appreciable impairment of growth was observed even after a 6 hour incubation period. The fact that the percentage of growth with the control as 100% was almost constant from 2 to 6 days after the removal of the irradiated sugar indicates that a certain fraction of the original cell population was damaged and/or probably killed. Those cells that for some reason survived the treatment continued to grow and multiply at the normal rate. Repair processes involve enzymes, and their efficiency depends on the time available between the production of the chemical change and the next replication of DNA.

The mutagenicity of irradiated media containing carbohydrates has been suggested by some investigators (Stone, 1955, and Swaminathan, 1963). It is well known that organic peroxides produced in the irradiated medium, together with formaldehyde and hydrogen peroxide, are quite potent mutagens. Peroxides have been found responsible for the mutagenic action of bacterial medium that has been exposed to heavy irradiation with ultraviolet light or ionizing radiation. In recent years, the sterilization of human food with high doses of ionizing radiation has given concern about the possible introduction of mutagens into human consumption.

Evidence for severe inhibition of the synthesis of DNA was obtained in the present work, especially when irradiated sucrose or fructose solutions were introduced to the growth medium of human amnion cells.

DNA and protein synthesis were demonstrated to be greatly diminished when rat liver slices were exposed to irradiated sucrose solutions by Aiyar and Sreenivasan (1969). They attributed the impairment of DNA synthesis to uncoupling of oxidative phosphorylation with a subsequent decrease in the formation of ATP.

The radiolysis of simple sugars has been extensively studied by Phillips and co-workers (1960, 1963). Among the products of radiolysis derived from sucrose in aqueous solution they reported: glucose and fructose as primary products, together with smaller amounts of glyoxal, glucosome and gluconic acid. Glucuronic acid, 2-oxogluconic acid, arabinose and 2- and 3-carbon aldehydic fragments arise in secondary processes. In addition, these authors have also reported the following compounds from the radiolysis of glucose: D-erythrose, formaldehyde, saccharic acid and 1:3-dihydroxyacetone. Hydrogen peroxide was also formed.

The presence of deoxycompounds in irradiated carbohydrates has recently been demonstrated by Scherz (1968). He has determined deoxycompounds originating from irradiated 1% solutions of sucrose

and glucose. The formation of polymers from glucose supposedly through gluconic acid has been reported by Barker et al. (1959). Hydroxyalkylperoxides derived from the interaction of radiolytic H_2O_2 with carbonyl compounds produced in the radiolysis of sucrose have proved to be toxic and mutagenic (Schubert, 1969). Perhaps a route different from that taken by Aiyar and Sreenivasan (1969) may lead to finding an answer for the marked inhibition of DNA synthesis observed in human cells under the effects of irradiated sucrose solutions.

The biosynthesis of DNA may be divided into three phases (Kihlman, 1966). The first of these is the de novo synthesis of uridilic acid (UMP), and inosinic acid (IMP). The second phase is the synthesis of the four deoxyribonucleoside triphosphates, which are the immediate precursors of DNA, and the third phase is the polymerization of these deoxyriboside triphosphates in the presence of DNA polymerase and a suitable DNA primer or template. From the second phase of the DNA synthesis, the formation of deoxycytidine-triphosphate (dCTP) is of importance for this discussion. The intermediate step of the reduction of cytidinediphosphate (CDP) to dCDP by CDP-reductase is strongly inhibited by a phosphorylated derivative of cytosine arabinoside, probably cytosine arabinoside diphosphate (Chu and Fischer, 1962). The product of the

CDP-reductase reaction, deoxycytidine diphosphate (dCDP) is then phosphorylated by a deoxyribonucleoside diphosphate kinase to the immediate DNA precursor dCTP. Arabinose is one of the radiolysis products of irradiated sucrose (Phillips, 1960). Cytosine arabinoside (CA) is the structural isomer of cytidine from which it differs by the configuration at carbon 2 in the pentose sugar. Kihlman et al. (1963) found that cytosine arabinoside induces chromosome breakage in human leukocytes at a concentration of 10^{-6}M. Although adenine deoxyribo-side (AdR), like CA, also inhibits the formation of deoxyribonucleotides, Chu and Fischer (1962) found that CA acts more specifically since apparently it is only the formation of deoxycitidine diphosphate from cytidine diphosphate which is inhibited by CA.

These reactions are of considerable interest, since they may provide a mechanism by which the supply of precursors needed for DNA biosynthesis is controlled.

SUMMARY AND CONCLUSIONS

Summary

1. The synthesis of DNA, RNA and protein in AV_3 cells was inhibited when the cells were incubated with 3 Mrad irradiated sucrose, glucose and fructose solutions. The average percentages of inhibition of DNA, RNA and protein synthesis (in that order) were: 49, 26, and 38 for sucrose; 20, 14, and 24 for glucose; and 51, 26, and 34 for fructose.

2. The synthesis of DNA in AV_3 cells was impaired most by sucrose and fructose.

3. The toxicity induced by the radiolysis products of sucrose and fructose was most accentuated at the dose level of 3 Mrad.

4. The synthesis of RNA and of protein in AV_3 cells was less affected than that of DNA in the same cells.

5. Irradiated glucose was less harmful to AV_3 cells in culture than sucrose or fructose.

6. Sucrose solutions irradiated at 3 and 4 Mrad and stored for 7 months at room temperature did not reduce the inhibitory effects on the synthesis of DNA in AV_3 cells.

7. A concentration of 40 μ g/ml of catalase only partly eliminated the deleterious effects of 3 Mrad irradiated sucrose to human amnion cells.

8. The activity of DNA polymerase from an AV_3 cells extract was not affected by the presence of irradiated sucrose in the substrate.

9. The treatment of irradiated sucrose with activated carbon did not remove the toxic material from the sugar.

10. Cell growth and reproduction was severely affected by incubation of the cells with 3 Mrad sucrose (5.8×10^{-2}M). The percentage of growth inhibition for the incubation periods of 6, 12 and 24 hours were 40, 60 and 90, respectively.

Conclusions

Under the conditions of these experiments it is apparent that the damage caused to human amnion cells by the radiolysis

products of sucrose and fructose cannot be repaired. The duration of the incubation of the cells with the irradiated sugar, the radiation dose applied, the pH of the medium and the stage of development of the cells are critical for their survival. It seems that only the fraction of cell population specifically involved in DNA synthesis (the S phase of development) was affected by the radiolysis products of the sugars. This effect is time dependent; the longer the incubation period, the higher the number of cells rendered unable to grow and multiply.

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APPENDIX

Table 6. --Effect of duration of incubation on the growth of AV_3 cells incubated with 3 Mrad irradiated sucrose solution (5.8×10^{-2}M).

Incubation time	Cells/ml		% Outgrowth
	Control	3 Mrad	
Enumeration of cells after 2 days			
6 hr.	2.31×10^6	1.41×10^6	61%
12 hr.	2.39×10^6	9.48×10^5	40%
24 hr.	2.42×10^6	2.68×10^5	11%
Enumeration of cells after 4 days			
6 hr.	3.51×10^6	1.98×10^6	56%
12 hr.	3.32×10^6	1.31×10^6	39%
24 hr.	4.09×10^6	3.24×10^5	8%
Enumeration of cells after 4 days			
6 hr.	4.43×10^6	2.68×10^6	60%
12 hr.	4.00×10^6	1.91×10^6	48%
24 hr.	3.79×10^6	3.83×10^5	10%

Table 7. --Percent inhibition of DNA synthesis in AV_3 cells incubated with 5.8×10^{-2}M sucrose, glucose and fructose solutions irradiated at 1, 2 and 3 Mrad (average; individual in parentheses).

Dose Mrad	Incubation time		
	2 Hours	4 Hours	6 Hours
	Sucrose		
1	20 (18, 16, 21, 25)	10 (12, 12, 7, 9)	20 (20, 25, 17, 18)
2	22 (17, 21, 25, 25)	32 (30, 34, 36, 28)	51 (47, 46, 53, 58)
3	68 (61, 66, 68, 77)	51 (52, 55, 48, 49)	53 (48, 50, 55, 59)
	Glucose		
1	8 (7, 9, 11, 5)	8 (8, 8, 10, 6)	32 (29, 35, 33, 31)
2	20 (21, 23, 17, 19)	11 (14, 8, 10, 12)	33 (31, 30, 38, 33)
3	25 (19, 27, 29, 25)	12 (9, 11, 17, 11)	33 (32, 33, 31, 36)
	Fructose		
1	20 (17, 21, 18, 24)	33 (30, 28, 36, 38)	39 (34, 37, 41, 44)
2	33 (29, 31, 37, 35)	40 (46, 39, 37, 38)	41 (38, 40, 40, 45)
3	52 (48, 51, 56, 52)	50 (52, 57, 46, 45)	63 (63, 68, 61, 60)

Table 8. --Percent inhibition of RNA synthesis in AV_3 cells incubated with 5.8×10^{-2}M sucrose, glucose and fructose solutions irradiated at 1, 2 and 3 Mrad (average; individual in parentheses).

Dose Mrad	Incubation time		
	2 Hours	4 Hours	6 Hours
	Sucrose		
1	12 (13, 8, 10, 16)	5 (3, 7, 5, 5)	9 (8, 13, 6, 10)
2	24 (30, 25, 28, 13)	17 (16, 18, 14, 20)	18 (20, 23, 14, 15)
3	29 (30, 25, 27, 34)	35 (30, 40, 37, 33)	24 (21, 20, 25, 30)
	Glucose		
1	8 (7, 10, 8, 7)	7 (9, 10, 5, 4)	10 (8, 12, 9, 11)
2	8 (6, 6, 9, 11)	15 (20, 15, 13, 12)	9 (13, 10, 9, 5)
3	22 (18, 20, 26, 24)	29 (31, 35, 26, 24)	13 (10, 10, 17, 15)
	Fructose		
1	2 (3, 2, 1, 2)	16 (14, 18, 20, 12)	4 (3, 2, 4, 7)
2	5 (4, 5, 7, 3)	30 (39, 40, 26, 35)	27 (24, 21, 33, 30)
3	19 (18, 15, 20, 23)	32 (33, 31, 28, 36)	27 (20, 27, 30, 31)

Table 9. --Percent inhibition of protein synthesis in AV_3 cells incubated with 5.8×10^{-2}M sucrose, glucose and fructose solutions irradiated at 1, 2 and 3 Mrad (average; individual in parentheses).

Dose Mrad	Incubation time		
	2 Hours	4 Hours	6 Hours
	Sucrose		
1	5 (4, 4, 7, 5)	29 (30, 34, 27, 25)	16 (13, 17, 20, 14)
2	26 (26, 29, 25, 24)	45 (40, 41, 49, 50)	27 (22, 26, 28, 32)
3	46 (41, 44, 49, 50)	52 (51, 47, 54, 56)	37 (32, 35, 37, 44)
	Glucose		
1	7 (8, 10, 5, 5)	23 (20, 19, 24, 29)	12 (11, 10, 10, 16)
2	12 (10, 12, 16, 10)	30 (29, 26, 31, 34)	8 (7, 6, 8, 11)
3	19 (15, 16, 21, 24)	36 (31, 33, 37, 43)	20 (18, 16, 23, 25)
	Fructose		
1	15 (19, 16, 12, 13)	15 (21, 15, 12, 12)	30 (37, 35, 26, 22)
2	20 (20, 23, 21, 22)	16 (10, 12, 21, 19)	30 (31, 36, 28, 25)
3	22 (18, 20, 25, 25)	18 (18, 23, 16, 15)	41 (39, 44, 46, 41)