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## The Effects of 2-Deoxyglucose on the ATP Consumption in Cell Cultures

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## The Effects of 2-Deoxyglucose on the ATP Consumption in Cell Cultures

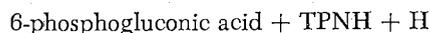
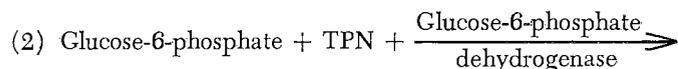
DARRELL D. YOUNG<sup>1</sup>

**SYNOPSIS:** Cultures of Chinese Hamster Cells (CCL 16, Don Strain) were treated with 2-Deoxyglucose over varying lengths of time in order to compare the mitotic index with the rate of adenosine triphosphate synthesis. Treatment with the glucose analog reduces both the mitotic index and the ATP level by nearly 50

percent. The relationship to the Swann "energy reservoir" hypothesis is also discussed.

**INDEX DESCRIPTORS:** 2-Deoxyglucose, mitotic index, adenosine triphosphate, cell cultures, Swann's "energy reservoir" hypothesis.

The primary purpose of this work was to determine if adenosine triphosphate (ATP) is necessary for cell division in mammalian cells. To achieve this purpose the glucose analog 2-deoxyglucose (2-DG) was used. It had previously been shown by Wick (1957), Kipnis (1959), Barban and Schulze (1961), Barban (1961, 1962) and Heredia (1964) that 2-DG inhibits glycolysis after it has been acted upon by hexokinase to form 2-deoxyglucose-6-phosphate. Hence 2-deoxyglucose-6-phosphate accumulates within the cell and effectively inhibits the utilization of glucose. The reactions which normally occur in the cell are given as follows:



Since the reduced TPN fluoresces, it is possible to measure the amount of ATP which is present.

Swann (1953) proposed that an "energy reservoir" existed in mammalian cells. This would imply that mitosis could continue, at least for a short time, if the production of ATP was curtailed or even stopped. However, later work by Epel (1963) has shown that CO inhibition in the eggs of the sea urchin, *Strongylocentrotus purpuratus*, resulted in a decrease in the normal ATP level along with a decrease in the rate of mitosis which stops completely when the ATP level drops below 50 percent of normal. This work, then, tends to disprove the "energy reservoir" hypothesis.

The work described within this paper was designed to further check the Swann "energy reservoir" hypothesis by using 2-DG as the inhibiting agent on Chinese Hamster Cells (CCL 16, Don Strain).

### MATERIALS AND METHODS

The cells were grown in monolayers in 906 media plus 10 percent fetal calf serum (FCS). Both 4 oz. and 16 oz. bottles were utilized. The 4 oz. bottles contained 15 ml. of media and were inoculated with 6 million cells each. These cells were used in making smears for determination of the mitotic index. The 16 oz. bottles contained 50 ml. of media and were inoculated with 20 million cells each. These cells were used for the analysis of ATP and protein.

Cells were collected by two means: 1) by use of trypsin

when smears were to be made, and 2) by use of 4 ml. of ice-cold 5 percent Perchloric Acid for the analysis of ATP and protein.

Smears were made from the trypsinized cells and stained with Wright's stain for 3 minutes followed with Giemsa stain for 30 minutes.

Extracts of cells treated with Perchloric Acid were neutralized with a saturated solution of K<sub>2</sub>CO<sub>3</sub> and then allowed to stand for 1 hour on ice. The assay of the extract for ATP was accomplished through a modification of the fluorimetric method of Greengard (1956), Table I. Also see Bergmeyer, ED., 1963.

The assay of the precipitate for protein was determined by means of the method developed by Lowry, et al. (1951).

TABLE I. MODIFICATION OF GREENGARD REACTION MIXTURE

Reagent	Conc.	Amt./3 ml.
Glucose	1.0 M	30 $\lambda$
TPN	5 x 10 <sup>-2</sup> M	40 $\lambda$
MgCl	0.15 M	30 $\lambda$
EDTA <sup>2</sup> (pH 7.4)	0.02 M	100 $\lambda$
Triethanolamine		
Buffer (pH 8.0)	0.1 M	350 $\lambda$
Sample	---	500 $\lambda$
Distilled H <sub>2</sub> O	---	to 3 ml.

**ATP Assay**—To the reaction mixture, which consists of a 500 $\lambda$  sample, is added the enzymes hexokinase and glucose-6-phosphate dehydrogenase in 40 $\lambda$  aliquots. The enzymes are diluted with distilled water in a 1:10 ratio. The ATP standards are established by adding ATP at 10<sup>-5</sup> M concentration in 50, 100, and 200 $\lambda$  aliquots. The instrument was set with 1 N H<sub>2</sub>SO<sub>4</sub>. The instrument used in the assay was an Aminco Fluorimeter, employing a primary filter Corning C-760 and a secondary filter Corning W# 75. The meter sensitivity was set to a maximum of 50 at a meter multiplier setting of .01.

**Protein Assay**—The protein was dissolved with 0.5 ml. of 1 N NaOH. 0.2 ml. of aliquots of the extract were mixed with 2 ml. of a reagent taken from a stock solution comprised of 50 ml. of 2 percent Na<sub>2</sub>CO<sub>3</sub> and 1 ml. of 0.5 percent CuSO<sub>4</sub>·5H<sub>2</sub>O in 1 percent in Na tartrate. This was allowed to set for 10 minutes after which 0.2 ml. of Folin's reagent was added and quickly mixed on a Vortex mixer. Samples were then allowed to stand for 30 minutes. All readings were taken on a Beckman-DB spectrophotometer at 750m $\mu$ . Standards were prepared from a stock solution of Ovalbumin with a concentration of 2 mg./ml.

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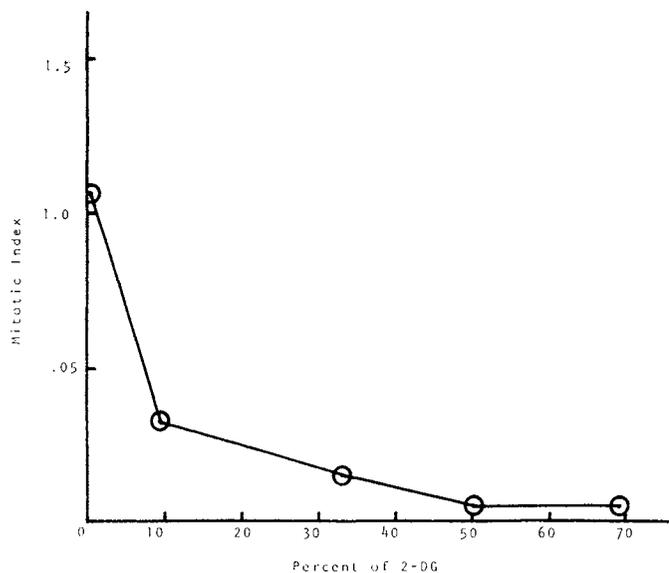


Figure 1. The effects of 2-DG on cells grown in varying ratios of 2-DG to glucose for 18-24 hours.

RESULTS AND DISCUSSION

Growth Effects—With varying concentrations of the 2-DG, growth of the cell line was markedly inhibited. As indicated by Figure 1, increased amounts of the 2-DG resulted in but slight changes in the mitotic index while initial amounts caused a rapid drop. The latter point is well illustrated by Figure 1, where it may be noted that a nearly 9 percent 2-DG in a 2-DG to glucose ratio resulted in approximately a 79 percent decrease in the mitotic index after 18 hours. For shorter periods of time the effects of the 2-DG on the mitotic index are illustrated in Figure 2. This shows that a rapid decrease occurs within the first 4 hours, followed by a 4 hour period of relative stability, after which a steady decline occurs.

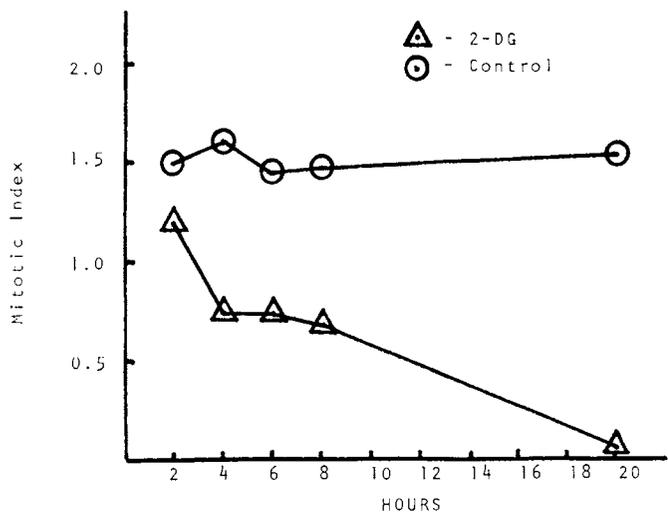


Figure 2. Effects of 2-DG on the mitotic index in terms of hours after exposure.

ATP—The ATP level, as found in the cell extract from cells grown in media containing 2-DG, apparently undergoes a rapid decline in amount as is indicated by the measurement of reduced TPN fluorimetrically, Figure 3. Figure 3 shows that after 8 hours the ATP level is approximately half that of normal. It may be assumed that this ratio would apply to the earlier readings although no controls were run for them.

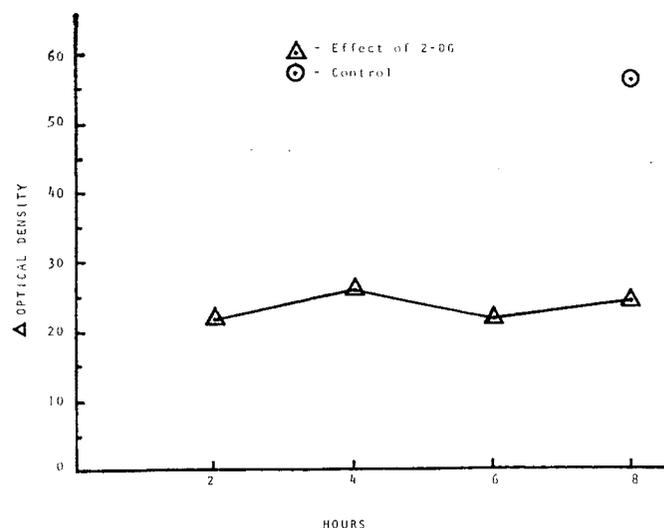


Figure 3. The level of ATP from cells grown in 906+10% FCS media containing 2-DG as indicated by the measurement of reduced TPN.

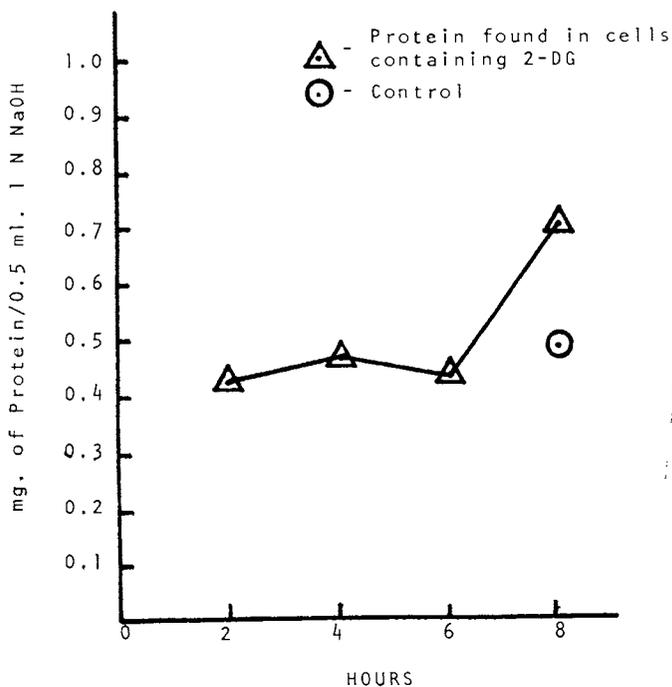


Figure 4. Amount of protein contained by cells exposed to 2-DG for varying time periods.

In relation to the ATP, the drop in the mitotic index corresponds closely (Figures 2 and 3). If the 2 hour level in both figures is examined closely it will be noted that while the mitotic index remains high, the ATP level has apparently been reduced to half or less. Except for this 2 hour level, the other values correspond. Therefore, it would seem logical to conclude that the 2-DG affects the ATP level rapidly but is not shown in the visual examination of the cells until approximately 4 hours after administration.

Protein—The amount of protein within the cells apparently remains rather constant as is indicated by Figure 4. The 8 hour level, however, is high as compared to the control and probably is not a true result. In relating the amount of ATP to protein (see Figure 5), it was found that after the 2-DG had been added the ATP level, as measured in  $\mu$  moles/140 mg. protein, remained relatively constant for 6 hours after which a decrease was noted. In comparison to the control, which was run at 8 hours, the level of ATP in the 2-DG exposed cells was half or less that of the control.

As a result of the preceding investigation, the following conclusions have been drawn:

- 1) The addition of 2-DG to cells reduces the ATP level to approximately one-half of normal.
- 2) The addition of 2-DG reduces the mitotic index to approximately one-half in 4 hours time.
- 3) The addition of 2-DG to cell cultures does not noticeably reduce the amount of protein present.
- 4) Mitosis can continue for a few hours when the ATP level is less than 50 percent.
- 5) Swann's hypothesis concerning an "energy reservoir" is further supported.

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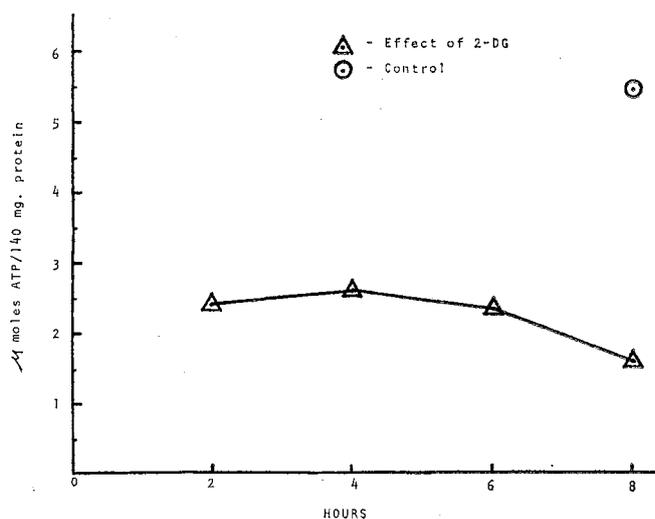


Figure 5. Calculated values of ATP in  $\mu$  moles/140 mg. protein.

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