Inhibition of Respiratory Activity of *Shigella sonnei* by Ultraviolet Irradiation.* (27655)

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Various workers have studied the effects of ultraviolet (UV) light upon the respiratory processes of bacterial cells. Giese(1) found that the respiration of *Achromobacter fisheri* cells exposed to low doses of UV irradiation was equal to that of the unirradiated control cells for a time, then the respiratory rate of the irradiated organisms declined. The period of "normal" respiration decreased as dosage of UV light was increased and the magnitude of the decline in respiratory rates increased directly with the UV dosage. Kelner(2) studied the effects of UV irradiation upon respiration of *Escherichia coli*. The UV dosage which he used resulted in almost total "death" of the cells as determined by colony-forming ability upon a suitable medium. Yet oxygen consumption of unirradiated and irradiated cells was comparable for a period which varied with the substrates employed. Heinmets and Katham(3) measuring the effects of UV irradiation upon bacterial metabolism and synthesis, employed a UV dosage which killed most of the cells. They found that oxygen uptake was suppressed in the presence of a nitrogen source but was only slightly inhibited in presence of glucose. When pyruvate was used as the substrate the oxidative capacity of the irradiated cells was lost for 2 hours; then there was a slight resumption of activity. On the other hand, irradiated cells took up oxygen at a slightly higher level than unirradiated cells in the presence of α-ketoglutarate and succinate. Billen, Stapleton, and Hollaender(4) exposed *E. coli* to X-irradiation and found that the numbers of cells capable of colony-formation after irradiation were about 0.05% of the population before irradiation. They observed a period of "normal" respiration in X-irradiated cells, followed by a decline in respiratory rate. The extent and duration of respiratory activity in the irradiated cells were less at 37°C than at 28°C. The period of respiration which equalled that of the control cells was longer when pyruvate and succinate were employed as substrates than when glucose was used. Sullivan(5) reported that the succinic dehydrogenase activity of irradiated and unirradiated *Mycobacterium lacticola* was comparable for a brief period of 15 minutes when the organism was irradiated for 10 minutes. However, when the cells were irradiated with UV light for 20-30 minutes, the enzyme activity level of the irradiated cells was lower than the unirradiated control cells.

The purpose of this present study was to determine the effects of UV irradiation upon respiratory activity of *Shigella sonnei*. We were primarily interested in the effects of UV dosage and the role of the substrate.

Materials and methods. *S. sonnei*, strain F-6, obtained from Dr. M. Finland, Boston City Hospital, Boston, Mass., was used throughout these experiments. The organism was grown in the chemically defined medium of Erlandson and Mackey(6) at 37°C for 20-22 hours. The cells were harvested, washed 3 times by centrifugation in 0.15 M phosphate buffer, and resuspended in buffer to yield a very dense cell suspension. Five ml of this suspension were pipetted into flat bottom petri dishes and irradiated. One ml aliquots of the cell suspension were pipetted, in duplicate, into aluminum planchets which had been weighed to the nearest 0.1 mg. The planchets were placed in a hot air oven at 80°C for 24 hours to dry the cells. One ml of the cell suspension weighed 13-20 mg.

The UV light source was a General Electric 15 watt germicidal lamp (G15T8) which

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emitted waves of approximately 2,537 Å. Under the conditions employed, the intensity of the UV irradiation was 44.2 ergs/sec/mm^2. The lamp was turned on for 20 minutes prior to use to allow it to equilibrate. The cell suspensions were shaken continuously during irradiation to obtain a uniform exposure of the cells to the UV light. The samples were irradiated for 7, 14, and 21 minutes, then their respiratory activity was measured employing glucose as the substrate. This was for the purpose of determining the effect of UV dosage upon oxygen uptake. In the experiments with varied substrates, the cell suspensions were irradiated for 10 minutes. Pyruvate, malate, α-ketoglutarate, succinate, furmarate, aspartate, serine, threonine, glutamine, and glutamate were employed as substrates. One ml aliquots of the irradiated cell suspensions were pipetted immediately into the main compartments of Warburg flasks which were already charged with 1.0 ml of 0.15 M phosphate buffer (pH 7) and 0.5 ml of substrate (0.05 M). Flasks without substrate served as endogenous controls. The respiration experiments were carried out at 37.2°C. Respiratory activity of *S. sonnei* was calculated and recorded as μl of oxygen consumed /10 mg dry weight of bacterial cells. Respiratory activity of unirradiated cells was also measured.

In some experiments cell suspensions were irradiated and then shaken with glass beads (Ballotini, 10A) in the Mickle tissue disintegrator for 1½ hours at 4°C. Respiratory activity of these disrupted cells was measured manometrically employing aspartate and pyruvate as substrates.

Results. The respiratory rate of *S. sonnei* was inhibited by UV irradiation when glucose was employed as the substrate (Fig. 1). The inhibition was directly related to the UV dose. UV treatment also inhibited endogenous respiration. Following 7 minutes of irradiation, the endogenous rate was inhibited approximately 25%. Irradiation for 14 and 21 minutes did not increase this inhibition. Table I shows the inhibition of respiratory rates when other substrates were employed by the irradiated cells. Inhibition ranged from 6% (fumarate) to 89% (aspartate) of the unirra-

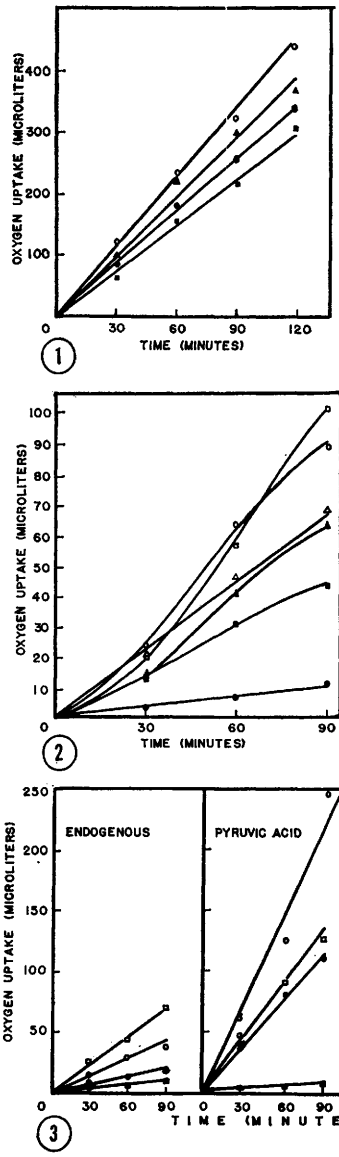

FIG. 1. Effect of ultraviolet dose upon respiratory activity of *Shigella sonnei*; substrate was glucose. ○—○ Control, ▲—▲ 18,564 ergs/mm^2, ●—● 37,128 ergs/mm^2, ■—■ 55,692 ergs/mm^2.

FIG. 2. Respiration of ultraviolet-irradiated and unirradiated *Shigella sonnei* employing different substrates. □—□ glutamic acid (unirrad.), ■—■ glutamic acid (irrad.), ○—○ aspartic acid (unirrad.), ●—● aspartic acid (irrad.), △—△ fumaric acid (unirrad.), ▲—▲ fumaric acid (irrad.).

FIG. 3. The respiratory activity of whole and disrupted unirradiated cells compared to the respiratory activity of cells which were disrupted after irradiation; undisrupted (whole) irradiated cells served as controls. ○—○ unirrad. whole cells, ●—● irrad. whole cells, □—□ unirrad. disrupted cells, ■—■ cells which were disrupted after irradiation.

TABLE I. Inhibition of Oxygen Uptake of *Shigella sonnei* by Ultraviolet Irradiation.

Substrate	% inhibition*	Substrate	% inhibition*
Fumarate	6	Pyruvate	61
Succinate	7	Glutamine	63
Glucose	11	Threonine	65
α-ketoglutarate	33	Serine	68
Malate	40	Aspartate	89
Glutamate	60		

* $100 - \frac{\mu l \text{ oxygen uptake by irrad. cells}}{\mu l \text{ oxygen uptake by unirrad. cells}} \times 100.$

Data represents oxygen uptake after 90 min employing cells which were irradiated for 10 min.

diated controls. Respiratory rates of the irradiated cells were lower than those of the unirradiated cells throughout the observation period. As a rule the respiratory rates were inhibited to a greater extent when amino acids were employed as substrates than when intermediates of the tricarboxylic acid cycle were employed. Fig. 2 illustrates the respiratory activities of *S. sonnei* when glutamate, aspartate, and fumarate were used as substrates. When the irradiated cells were mechanically disrupted there was almost a complete loss of respiratory activity (Fig. 3).

Discussion. Although many workers have reported that the respiration of irradiated cells paralleled that of unirradiated cells for a period and then gradually declined, in our experiments the respiratory rate of the irradiated cells was lower than the unirradiated cells throughout the observation period. This discrepancy may have been due to differences in the organisms employed or UV dosage used. Swenson and Giese(7) stated that adaptive enzyme formation was inhibited by irradiation. Billen *et al.*(4) and Brandt, Freeman, and Swenson(8) hypothesized that the inhibition of new enzyme synthesis (due to irradiation) rather than the destruction of preformed enzymes was responsible for the inhibition of oxidative activity in irradiated cells. Giese(1) believed that a period of normal activity present in irradiated cells was due to the preformed enzymes, and that the gradual decline in respiratory activity was a result of inability to synthesize new enzymes as a result of irradiation. On the other hand, the work of Sullivan(5) and our work have shown that there was almost immediate inhibition in the irradiated cells indicating damage to the preformed enzymes. We cannot determine from these data whether the inhibition of respiratory activity was due to inhibition of all of the cells or to greater inhibition of some of the cells exposed to irradiation. One possible explanation for the loss of respiratory activity in disrupted cells is that the UV treatment may have sensitized the preformed enzymes so that they were more easily damaged by mechanical shaking.

The oxidation of amino acids was unusually sensitive to irradiation. Transamination reactions have been reported in *S. sonnei.* Sen (9) reported that *Shigella* converted glutamic acid to α-ketoglutaric acid. Aspartic acid was deaminated to yield fumaric acid and ammonia. In our studies there was greater inhibition of oxygen uptake when glutamic and aspartic acids were used as substrates than when α-ketoglutarate or fumarate were used. If the total respiratory activity of *Shigella* was a result of a deamination of the amino acids (glutamic and aspartic) and subsequent oxidation of α-ketoglutarate and fumarate *via* the tricarboxylic acid cycle, then it is possible that the greater inhibition of oxygen uptake when amino acids were employed as substrates was due to inactivation of the transaminating enzymes by UV light. Another possible explanation for the different levels of inhibition with different substrates is that the turnover rates of the various enzymes may differ. Therefore, even though the sensitivity of the enzymes to irradiation may be equal, the destruction of enzymes with high turnover numbers would result in a greater decrease in oxygen uptake than would the destruction of enzymes with low turnover numbers.

Summary. 1. The respiratory activity of *Shigella sonnei* was inhibited by UV irradiation. 2. A direct relationship existed between UV dosage and extent of inhibition. 3. The inhibition of respiratory activity varied considerably with different substrates. 4. Irradiated cells which were disrupted by mechanical shaking with glass beads lost practically all respiratory activity.

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Possible Factor Produced During Muscular Contraction which Influences the Passage of Glucose.* (27656)

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Muscular work increases glucose utilization by muscle possibly by a mechanism which regulates the permeability to glucose. Sacks and Smith(1) have shown that there is a specific steric requirement for the non-metabolized sugar to penetrate the cell membrane during muscular contraction. The effects of insulin and muscular work are additive. Goldstein and co-workers(2,3) have suggested that this increased permeability for glucose could be due to a humoral effect.

Our experiments have been performed to determine if a buffer solution which has been surrounding a contracting uterine muscle, affects the passage of glucose to diaphragm, adipose tissue of the epididymis, kidney and brain.

Materials and methods. Buffer solutions surrounding uteri in periodic contraction and permanent relaxation were obtained as previously described(4). The effect of buffer (C-B) used for contracting tissue on glucose uptake was measured by diluting it in Warburg flasks with Krebs-Henseleit buffer. When diaphragm was used, 1 ml of C-B was mixed with 1 ml of Krebs-Henseleit, when kidney, epididymal fat or brain were employed, 0.1 ml of C-B was mixed with 1.9 ml of Krebs-Henseleit. Pyruvate was also added to the latter when brain tissue was incubated. Equal volumes of "relaxation buffer" (R-B) were used for the controls. Incubation conditions were as follows: the final volume was 2 ml, glucose concentration was 3 mg/ml, the wet weights of brain slices were 50-75 mg, those of kidney, diaphragm and epididymal fat 150-200 mg. Incubation lasted 1½ hours for muscle and kidney, 1 hour for brain and 4 hours for epididymal fat and was carried out at 37°C with 70-80 cycles of 2 cm amplitude/min in air, except for brain tissues. In the latter case a 95% O_2-5% CO_2 mixture was used. Glucose was determined by the Somogyi-Nelson method and glucose uptake is expressed as mg of glucose disappearing from the medium/100 mg tissue (wet weight). Oxygen consumption is given as μl/hr/100 mg tissue (wet weight). Statistical analysis of results was performed using the paired observations method (Diaphragm, adipose tissue and kidney) or "t" method of Student (5), for brain.

Results and discussion. The buffer previously used for incubation of contracting uterine muscle increases the permeability of diaphragm, epididymal fat and kidney to glucose (Table I). The sensitivity of these tissues to the supposedly humoral factor varies. Adipose and renal tissue are 10 times more sensitive than the striated muscular tissue (diaphragm). Glucose uptake of the brain does not seem to be influenced, though respiration increases significantly.

Our results support the hypothesis that a

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