SUMMARY

The intracellular distribution of amylase in rat pancreas has been studied by differential centrifugation of pancreas homogenates. Although the secretory granules showed high activity, at least half the amylase activity was recovered in the microsome and supernatant fluid fractions. In the latter fractions, the proportion of the total enzyme activity and the concentration of amylase activity relative to protein-nitrogen showed consistent variations with changes in the secretory state of the gland. Apparently some of the amylase is associated with the endoplasmic reticulum in the living pancreas cell, and during homogenization in 0.88 *M* sucrose the endoplasmic reticulum is broken into pieces resembling one another in composition but differing in size. During exposure to 0.25 *M* sucrose some dissociation of the components of this material takes place, but when such dissociation occurs the amylase protein is not preferentially associated with either the ribonucleoprotein granules or the lipoprotein reticulum of the microsome fraction.

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SYNTHESIS OF NUCLEIC ACIDS IN ULTRAVIOLET-TREATED *ESCHERICHIA COLI**

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Starvation increases the sensitivity of protozoa[1,2] and of yeast (unpublished observation) to ultraviolet (UV) radiations, in the latter case the absence of nitrogen compounds being especially effective in this respect. On the other hand DEMEREC AND LATARJET[3] have shown that *Escherichia coli* B/r is 2–3 times more resistant to UV in the resting than in the growing state, and WITKIN[4] using the same strain showed that the bacteria are more resistant to UV in the lag phase of a culture than in any other phase of the growth cycle.

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As a basis for further analysis of the relation between nutritional and growth state of a cell and its resistance to UV, an investigation of the sensitivity of *E. coli* B in logarithmic (log) phase and just out-of-log phase was carried out. Particular attention was directed towards the possible mechanism of action of UV upon the cell. Since experiments in this direction have previously demonstrated that deoxyribose nucleic acid (DNA) synthesis is one of the primary loci of action of UV[5], a study was made of DNA synthesis before and after UV treatment. The effect of UV on ribose nucleic acid (RNA) synthesis was also studied, because it might be altered in out-of-log phase cells even though it seems to be unaffected in the log phase cultures tested by KELNER[5] and others[6,7,8]. The data show a marked effect of UV radiation on the synthesis of RNA in out-of-log phase *E. coli*. Action of UV on a function in the cell other than nucleic acid synthesis is also postulated on the basis of studies with UV radiations of very short wavelength (226 mμ) upon growth and nucleic acid synthesis in out-of-log phase *E. coli* B cultures.

MATERIALS AND METHODS

Cultures of *Escherichia coli* B, obtained from the Medical Microbiology Department at Stanford University, were maintained by weekly transfers on agar slants of the medium described by DAVIS AND MINGIOLI[9]. Agar was omitted from this medium for liquid cultures of the bacteria.

Bacteria in the log phase were obtained by inoculating 200 ml of nutrient medium contained in a 500 ml Ehrlenmeyer flask with a loopful of bacteria scraped from a fresh slant, and incubating the culture on a shaker for 16 hours at 30° C. At the end of this time a 50 ml aliquot of the suspension was withdrawn and used to inoculate another flask containing 200 ml of medium. After two hours growth under the same conditions, a 60 ml aliquot of the culture was withdrawn for experimental use. Checks, made by plating samples from the culture at various times, showed that the bacteria were in log phase at this time.

To obtain bacteria in the out-of-log state, cultures were inoculated in the same manner as above, but they were grown for 20 hours under the same conditions, at which time plate counts showed them to have been in the out-of-log phase for 5 hours. A suspension of the bacteria was then centrifuged down, washed once in 100 ml of phosphate buffer[9], and resuspended in the buffer.

Determination of number of cells per ml was made by diluting sufficiently, with buffer medium, an aliquot of the suspension in each culture flask to give about 100 colonies per plate. Preliminary trials with a series of dilutions established the appropriate range of dilution required. All samples, plated in triplicate, were counted after 3 days incubation in the dark at 27° C.

Two sources of radiation were used in these studies. A quartz mercury arc running at atmospheric pressure was used in some experiments, the radiations being passed through a natural quartz monochromator. In this case experiments were performed with wavelengths 226 and 265 mμ, the output of the arc at each wavelength being measured with a thermopile calibrated against U.S. Bureau of Standards Standard Lamps. Other experiments were performed using a G.E. Sterilamp that gives off radiations principally at 254 mμ, the intensity of which was measured by a Hanovia UV meter. Experimental dishes, in the latter case, were placed at a distance at which the UV intensity was 40 ergs/mm^2/sec.

Ultraviolet light injury of the cells was photoreversed by illumination with white light (WL) from two 90 watt G.E. daylight fluorescent lamps 31 cm from the surface of the bacteria suspension, an exposure of 45 minutes being used in all cases. The bacteria, suspended in nutrient medium were put under the fluorescent lamps within 10 minutes after being placed in the nutrient medium, since bacteria in nutrient medium soon lose their ability for photoreversal (KELNER[10]).

Escherichia coli suspensions for irradiation purposes were prepared in either of the two following ways. In experiments on log phase cells a 12 ml aliquot of the log phase culture was added into each of 5 Petri dishes (9 × 1.5 cm), and during irradiation under the Sterilamp each dish was continuously swirled by hand to insure uniform irradiation of the bacteria. Following irradiation the contents of the 5 Petri dishes were pooled and an aliquot of this suspension was inoculated into two flasks, each containing 100 ml of nutrient medium: the initial population of the controls derived from the same stocks as the experimentals was found to be $2 \cdot 10^8$ bacteria per ml. After treatment (one culture being subjected to visible light after UV), the controls, UV-irradiated, and UV-irradiated + WL-illuminated cultures, were grown at 34° C, being aerated by agitation with glass-enclosed magnetic fleas. Periodic plate counts and determination of the nucleic acid content were made.

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In experiments on out-of-log phase bacteria, a 14 ml aliquot of the washed suspension in buffer was poured into a sterile quartz Petri dish (5 × 1.2 cm) and exposed to UV light from the monochromator (226 or 265 mμ). During irradiation the suspension was stirred continuously with a glass-enclosed magnetic flea. Following irradiation, aliquots from the control, the UV-irradiated, and the UV-irradiated + the WL-illuminated suspensions, were each inoculated into separate flasks containing nutrient medium, the aliquot being of a volume to give an initial cell count of $2 \cdot 10^8$ bacteria per ml in the controls. They were incubated at 30° C under constant aeration. Plate counts and periodic determinations of nucleic acid were made.

All cultures during and after irradiation and illumination were handled in a room lighted by 40 watt ruby lamps, and they were grown in the dark.

Nucleic acids were extracted using the HERSHEY, DIXON AND CHASE[11] adaptation of the SCHMIDT AND THANNHAUSER[12] and SCHNEIDER[13] procedures. A 10 ml sample of suspension from each of the flasks was used for each determination—from control, UV-irradiated, and UV-irradiated + WL-illuminated suspensions. The sample to be tested was added to a test tube (15.5 × 1.5 cm) in which all extractions were performed. From the centrifuged cells, phospholipids were first removed from each sample by adding 5 ml of 95% ethanol and ethyl ether (3:1) to the mixture and heating for 5 minutes to the boiling point of the mixture, centrifuging, and pouring off the supernatant fluid. The precipitate remaining after pouring off the ethanol-ether, was washed once with 5 ml of 0.3 *M* trichloracetic acid (TCA).

RNA was extracted from the precipitate by hydrolysis in 1 ml of 1 *N* NaOH for 18 hours at 20–24° C, at the end of which time, 1 ml of 1 *N* HCl and 0.2 ml of 3 *M* TCA were added to precipitate the DNA-protein. To the supernatant fluid, containing the hydrolysate of RNA, poured into a separate tube was now added a subsequent 1 ml of 0.3 *M* TCA washing of the DNA-protein precipitate. To a 1 ml aliquot of the RNA extract was added 0.15 ml of 10% orcinol (MacKay Brand, recrystallized twice from benzene) and 2.5 ml of 0.033% $FeCl_3$ dissolved in concentrated HCl[14]. The tubes, capped with glass marbles and shaken, were placed in boiling water for 20 minutes. After cooling under running tap water the optical density of the solution at 565 mμ was determined with a Beckman spectrophotometer. Experiments were performed in a manner such that duplicate experimental and control determinations with the spectrophotometer could be completed within 30 minutes from the time they were removed from the boiling water bath. The RNA standards (Delta Chemical Works, New York) and the blank were made up in 0.3 *M* TCA.

The DNA was extracted from the DNA-protein precipitate following the removal of the RNA (as described above) by two successive extractions at 90° C for 15 minutes with 1.5 ml of 0.3 *M* TCA in each case, the supernatant fluid being collected in another tube for testing. The indole method of DISCHE[15] as modified by CERIOTTI[16] was used to determine DNA, with the exception that the samples used were just half as large, since 0.5 ml micro-cuvettes (Pyrocell Mfg. Co., N.Y.) were used. The DNA standard (Schwarz Lab., Inc., New York, N.Y.) and the blank were made up in 0.3 *M* TCA.

EXPERIMENTAL RESULTS

1. *Comparison of effects on log and out-of-log phase cultures*

The effect of a dose of 1000 ergs/mm² of UV (254 mμ) radiations from the Sterilamp on growth and nucleic acid content of *E. coli* B cultures irradiated in the log phase is shown in Fig. 1. Such a dose reduces the survivors to 0.5% of the initial population. Because of the nature of treatment just before and during irradiation, a lag phase of about 70 minutes after zero time (time of irradiation) precedes logarithmic growth after inoculation of the controls into the nutrient medium. In the UV-treated culture the log growth phase begins in 120 minutes and the UV-treated + WL-illuminated cultures in 100 minutes. In all three cultures synthesis of RNA begins, or is proceeding, at zero time. Synthesis of RNA in the controls continues until the end of the log phase, while in UV-irradiated and UV-irradiated + WL-illuminated cultures RNA synthesis declines after 35 minutes, commencing again when cell division begins. Thereafter, RNA synthesis is seen to parallel the respective growth curves. Synthesis of DNA begins soon after zero time in controls and continues thereafter to parallel the growth curve. In the UV-treated cultures, however, DNA synthesis is inhibited at zero time and begins again only when growth does; that is, when increase in number of bacteria is observed.

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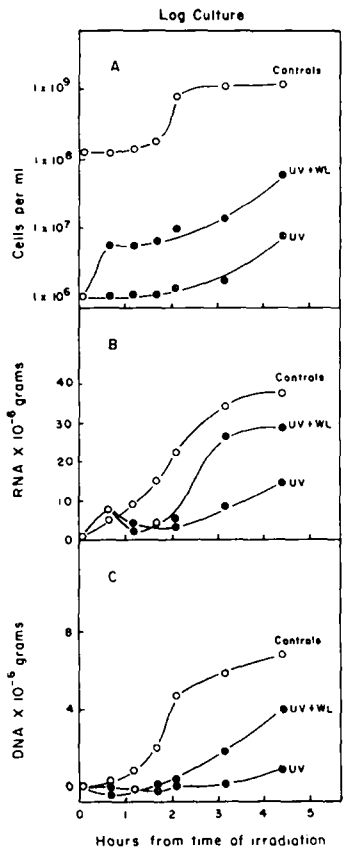

Fig. 1.

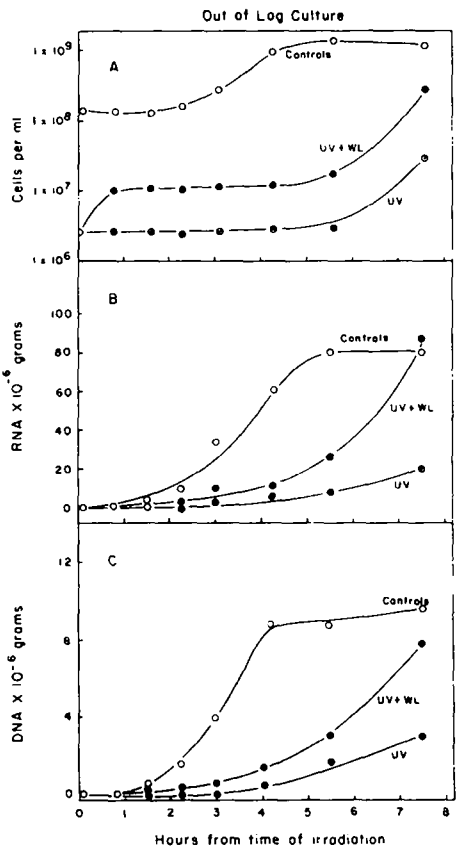

Fig. 2.

Figs. 1 and 2. Comparison of effects of irradiation with UV of λ 254 mμ on *Escherichia coli* cultures in the logarithmic phase of culture (Fig. 1) with those out-of-log phase (Fig. 2). In A of each figure is given the number of cells per ml in control and irradiated cultures, in B, the content of RNA per 10 ml aliquot of the suspension of bacteria, and in C, the content of DNA per 10 ml aliquot of the suspension of bacteria after the end of irradiation (zero time).

The results of a similar series of experiments using the same dose of 254 mμ on out-of-log phase cells is given in Fig. 2. This dosage (1000 ergs/mm^2) reduces the viable population to 2.5% of the initial number. In the controls the DNA and RNA per cell increases about twofold during the lag phase; thereafter the increase in total DNA and RNA parallels the increase in numbers. Synthesis of RNA begins in the UV-irradiated + WL-illuminated culture next and last in the UV-treated ones, and thereafter the synthesis of RNA in each culture parallels the respective growth curve. Although the out-of-log *E. coli* are more resistant to UV than log cultures, the delay in the initiation of RNA and DNA synthesis by UV is greater in the out-of-log cells than in the log cells.

2. *Comparison of effects of short (265 mμ) and very short (226 mμ) ultraviolet radiations*

The purpose of these experiments was to determine what effects very short UV radiation (226 mμ), which is very superficially absorbed, would have on growth and

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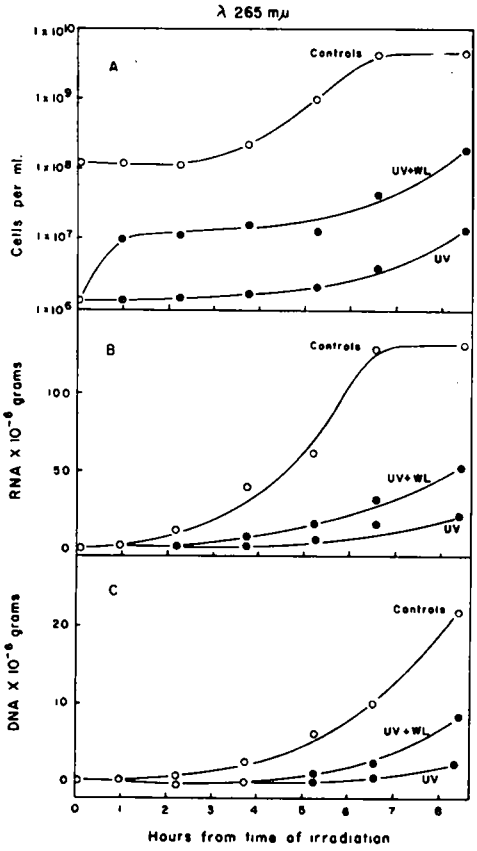

Fig. 3.

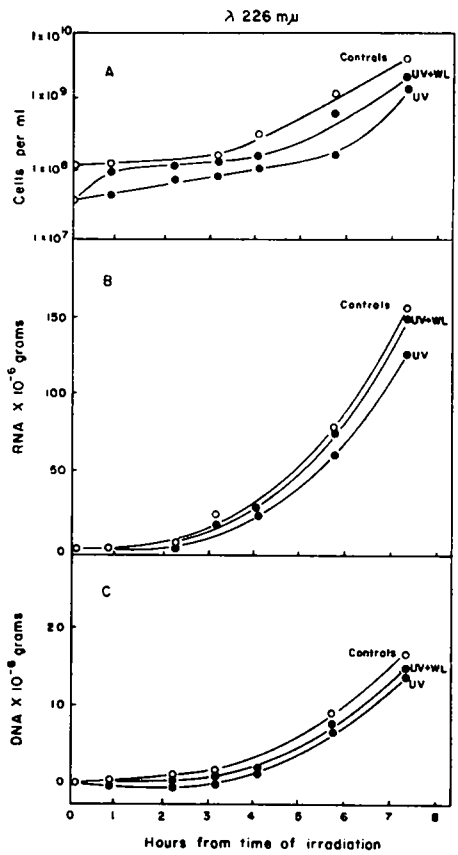

Fig. 4.

Figs. 3 and 4. Comparison of effects of irradiation with UV of λ 265 mμ in Fig. 3 with that of λ 226 mμ in Fig. 4. In A in each case is given the number of cells per ml in control and irradiated cultures, in B, the content of RNA per 10 ml aliquot of the bacterial suspension, and in C, the content of DNA per 10 ml aliquot of the bacterial suspension. All cultures in Figs. 3 and 4 are out-of-log phase.

nucleic acid synthesis in comparison to the effects of a longer wavelength (265 mμ) which penetrates deeper into the cell. Since more information seemed to be obtained from the use of the out-of-log cultures of *E. coli* in the preceding experiment than from the log phase cultures, the former only were used here. Since a dense suspension of bacteria in buffer had to be used in the exposure cell as only a small volume could be covered by the small beam of UV radiation available from the monochromator, no cell was continuously irradiated even though the suspension was continuously stirred. The dose was adjusted by preliminary experiments to give about the same proportion of survivors, 2.5%, as in the first series of experiments described above. At 265 mμ this took about 45 minutes of exposure and the incident dose was 6000 ergs/mm^2, but presumably each cell had a much smaller dose. At 226 mμ the dose was 3000 ergs/mm^2 and a survival of 50%.

The results are shown in Fig. 3 for experiments with UV at wavelength 265 mμ. In the controls, synthesis of RNA and DNA begins before cell division, but in the

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treated cells it is delayed, commencing about the time the number of cells increases in each culture, the results being essentially the same as those described for the out-of-log cells in the series of experiments irradiated with the Sterilamp.

In Fig. 4 are shown the results for experiments with UV of wavelength 226 mμ. A longer lag phase of growth than in the control is observed for the UV-irradiated, and UV-irradiated + WL-illuminated cultures; the lag is relatively small compared to that which occurs at the somewhat longer wavelengths (*e.g.* 265 mμ). Growth is therefore less affected by radiations of this wavelength than by the longer wavelengths (254, 265 mμ). Much more striking is the lack of almost any effect of the very short UV radiations (226 mμ) upon nucleic acid synthesis, the curves practically paralleling the controls.

DISCUSSION

Two findings reported in this paper require amplification and discussion: (1) the reduction of RNA synthesis along with a similar effect on DNA synthesis in *E. coli* when the out-of-log phase cultures are treated with short UV (254, 265 mμ); (2) the almost complete lack of effect of very short UV (226 mμ) upon nucleic acid synthesis in *E. coli* in spite of the injurious effects of these radiations on its viability.

The decrease in RNA synthesis along with DNA synthesis of the out-of-log phase *E. coli* may account for the greater resistance of such cells to UV. In log phase cells DNA synthesis is reduced or stopped by UV, but RNA synthesis and protein synthesis continue for a period of time. Unbalanced growth resulting from continued increase in protein and RNA may possibly be the cause of injury and death[17]. The greater sensitivity of the log phase *E. coli* B/r than of bacteria from other phases in the growth cycle[3,4] and in the present paper for *E. coli* B might be accounted for in this manner. It would be desirable to determine the synthesis of RNA, DNA, and protein after irradiation of bacteria in still other phases of the growth cycle than those tested here.

Since synthesis of RNA in *E. coli* B is not as readily inhibited by irradiation with short UV (254 and 265 mμ) in the log phase cultures as in the out-of-log cultures, two loci of RNA synthesis are suggested, one in the cytoplasm and one in the nucleus. The locus of RNA synthesis in the cytoplasm presumably predominates in the log phase cultures and is more resistant to UV than the locus in the nucleus, which predominates in the out-of-log phase cultures. Data on *Amoeba* show even more clearly that RNA is synthesized in both nucleus and cytoplasm[18], the synthesis in the nucleus proceeding sooner than in the cytoplasm after removal of RNA by ribonuclease[19].

DNA synthesis is equally sensitive to UV during both phases of growth of *E. coli* studied here. Presumably only one synthetic locus is present—in the nuclear components.

Killing and retardation of division of *E. coli* following irradiation with wavelength 226 mμ shows that the radiations damage the cells but the survivors show no change in synthesis of RNA and DNA. It is possible that such short radiations (226 mμ) do not penetrate deeply enough inside the cells to affect the "nuclear" constituents, because they are so superficially absorbed by protoplasm[20], therefore they fail to affect nucleic acid synthesis. On the other hand, photoreactivation from

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injury caused by such very short UV radiation occurs after illumination, and since effects of UV primarily localized in the nucleus are readily photoreversed whereas those localized in the cytoplasm are not[21,22], one would be inclined to argue that in *E. coli* the "nucleus" had also been reached by UV of wavelength 226 mμ, since photoreactivation of UV injury by visible light was found to occur. Therefore, a locus of action of the UV radiations other than the part of the cell concerned with nucleic acid synthesis is suggested by these data, although the location of such a site is not evident.

SUMMARY

1. The effect of various wavelengths of ultraviolet (UV) radiation upon the synthesis of nucleic acids in cultures of *Escherichia coli* B in logarithmic (log) and out-of-log phases of the growth cycles was studied.

2. Treatment of cultures of log phase *E. coli* B with UV radiation of 254 mμ stops desoxyribonucleic acid (DNA) synthesis immediately, whereas ribonucleic acid (RNA) synthesis continues for about one division cycle (30 minutes) afterwards.

3. Treatment of out-of-log phase cultures of *E. coli* B with these radiations stops DNA and RNA synthesis immediately, as does treatment with UV of 265 mμ.

4. After irradiation with UV of λ 226 mμ the synthesis of RNA and DNA is not delayed as compared to the controls.

5. RNA and DNA synthesis, stopped by UV, is resumed first in the cultures subsequently illuminated with white light, indicating photoreversal of UV injury by white light, and then in the UV-treated cultures. In both cases, synthesis of nucleic acid is resumed at about the time that the bacteria begin to divide.

6. Since RNA synthesis is inhibited by UV (254 mμ or 265 mμ) in *E. coli* cultures in the out-of-log phases of the growth cycle but not in the log phase cultures, two loci of action of UV radiations on RNA synthesis are suggested. Citations supporting this view are discussed.

7. The decline in viability of *E. coli* B after irradiation with very short UV (226 mμ) without a corresponding decrease in RNA and DNA synthesis suggests a locus of action of these radiations on the cell different from the part of the cell involved in nucleic acid synthesis.

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