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Evidence for the Lack of Deoxyribonucleic Acid Dark-Repair in *Halobacterium cutirubrum*

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1. *Halobacterium cutirubrum* does not perform dark-repair of DNA either after u.v. irradiation or during normal growth. 2. Cultures irradiated with u.v. are readily photoreactivated, but do not recover viability in the dark. 3. No increase in the rate of DNA synthesis is observed in the surviving cells after u.v. irradiation. 4. At early times during normal semiconservative replication, newly incorporated thymidine is found only in the hybrid DNA. 5. It is suggested that these bacteria may be useful in the study of DNA replication and photoreactivation.

The principal mechanisms used by living cells for the repair of damage to their DNA are photoreactivation and dark-repair (Setlow, 1968; Setlow & Setlow, 1972; Howard-Flanders, 1973). The latter process, found in most well-studied cells, involves removal of the damaged sections of the genome and synthesis of new material. Occurrence of repair biosynthesis of DNA greatly complicates study of DNA replication, since it cannot be assumed that the precursors or enzymes required for the two processes are necessarily the same (Werner, 1971; Harris, 1973). Clayton *et al.* (1974) found that mitochondria lack DNA excision–repair, but the extent to which DNA replication in these organelles mimics that in the nucleus or in prokaryotes is uncertain, so a wild-type cell totally lacking dark-repair would be a useful tool.

Hescox & Carlberg (1972) found that the extreme halophile *Halobacterium cutirubrum* has an efficient photoreactivating system. They also stated, but without presenting any evidence, that the u.v.-irradiated cells did not recover viability in the absence of light; this suggested to us that *H. cutirubrum* might lack dark-repair and thus be a promising organism in which to study DNA replication. We now present detailed proof that dark-repair and its associated DNA biosynthesis do not occur in this organism.

Experimental

Materials

Enzymes, chromatographic supplies, reagents and substrates were purchased from the following suppliers: radioactive compounds and PCS liquid-scintillation counting fluid, Amersham/Searle Corp., Oakville, Ont., Canada; MN Polygram 300 plastic-backed cellulose t.l.c. sheets, Brinkmann Instruments (Canada) Ltd., Rexdale, Ont., Canada; *Streptomyces griseus* proteinase (Pronase), Calbiochem, La Jolla, CA, U.S.A.; Difco agar, casamino acids and yeast extract, Canlab, Montreal, P.Q., Canada; Schwarz–Mann CsCl for density-gradient centrifugation, Picker X-Ray Engineering, Ottawa, Ont., Canada; non-radioactive nucleosides, nucleotides and thymine, P–L Biochemicals, Milwaukee, WI, U.S.A., or Sigma Chemical Co., St. Louis, MO, U.S.A.; amino acids, proteinase-free pancreatic RNAase* A (type I-A) and electrophoretically purified pancreatic DNAase I, Sigma Chemical Co. All other chemicals were obtained from Fisher Scientific Co., Ottawa, Ont., Canada, and were analytical reagent grade.

Methods

Cell culture. Stock cultures of *H. cutirubrum* N.R.C. 34001 (formerly strain no. 9) were maintained on slants of Gochnauer & Kushner's (1969) complex medium containing 2% (w/v) agar, as previously described (Peterkin & Fitt, 1971).

Liquid cultures were grown in sterile conditions in culture flasks fitted with stainless-steel closures. Incubation was in a New Brunswick Aquatherm gyratory water-bath shaker at 250rev./min and 37°C. In all cases inocula were 10% (v/v) relative to the volume of fresh culture medium.

The growth of liquid cultures was followed by determining the number of viable bacteria and/or by measuring the turbidity of the bacterial suspension at 660nm relative to aq. 2% (w/v) NaCl with a Zeiss PMQ II spectrophotometer. Viable cell counts were performed in sterile disposable plastic Petri dishes (10cm diam.) containing 2% (w/v) agar in Gochnauer & Kushner's (1969) complex medium (15ml).

* Abbreviations: RNAase, ribonuclease; DNAase, deoxyribonuclease.

Samples (0.1 ml) of serial dilutions of the culture in sterile 25% (w/v) NaCl were spread over the surface of the nutrient agar. The dishes were placed in plastic bags to prevent desiccation and incubated at 37°C in a moist atmosphere. Colonies were counted after 5–7 days until no more appeared, and each dilution was assayed in duplicate.

All incorporation studies were performed in the synthetic culture medium of Grey & Fitt (1976). Additions to this medium are listed in the descriptions of the appropriate experiments.

Irradiation experiments. Cultures of *H. cutirubrum* in the synthetic medium supplemented with 82.6 μM-thymidine were grown at 37°C and 250 rev./min until the cell density was approx. 10^9 cells/ml. Samples (15 ml) of the cell suspension were transferred to an uncovered sterile Petri dish placed on the platform of a Lab-line Junior Orbit gyratory shaker (Canlab, Montreal, P.Q., Canada). The culture was swirled gently (100 rev./min) at room temperature (25°C) and irradiated for the indicated times with a u.v. lamp (Mineralight, Ultra-Violet Products, San Gabriel, CA, U.S.A.) suspended 24–27 cm above it. The dose rate in ergs/mm^2 per s was measured with a Black-ray u.v.-meter (Ultra-Violet Products). Survival curves were determined by withdrawing samples (0.1 ml) at suitable intervals with sterile pipettes; they were then diluted serially with 25% (w/v) NaCl for measurement of the viable cell count.

Photoreactivation experiments. H. cutirubrum cells were irradiated as described above and samples (0.1 ml) were withdrawn immediately before and after irradiation for determination of the viable cell count. The Petri dish containing the remainder of the irradiated culture was placed on ice 10 cm below two white fluorescent desk lamps (General Electric F6T5-D) and irradiated while the culture was swirled gently (100 rev./min) on a Lab-line Junior Orbit shaker. Samples (0.1 ml) were withdrawn at intervals for determination of the viable cell count.

Detection of semiconservative replication. A culture flask containing the synthetic medium supplemented with 68 μM-5-bromo-2′-deoxyuridine was inoculated with 5 ml of a culture of *H. cutirubrum* previously grown to a cell density of approx. 10^{10} cells/ml in the synthetic medium supplemented with 82.6 μM-thymidine. After 48 h of growth at 37°C, 2.5 ml of this culture was transferred to fresh synthetic medium (25 ml) containing 68 μM-5-bromo-2′-[6-^{3}H]deoxyuridine (sp. radioactivity 3.7 Ci/mol), and incubation was continued for a further 31 h. A sample (2 ml) was then withdrawn for treatment as described below and the remainder of the culture was centrifuged at 45000$g_{max.}$ for 5 min. The cells were resuspended in preincubated synthetic medium (25 ml) containing 82.6 μM-[2-^{14}C]thymidine (sp. radioactivity 0.61 Ci/mol). Incubation was continued at 37°C, and samples (2 ml) were withdrawn immediately after resuspension of the cells (zero time) and at 60 min (0.125 generations), 8 h (1 generation) and 16 h (2 generations). The samples were added to 25% (w/v) NaCl (10 ml) and the cells were immediately sedimented at 45000$g_{max.}$ for 2 min. The pellets were washed once by resuspension of the cells in 25% (w/v) NaCl (10 ml) followed by centrifugation, and the washed cells were lysed carefully by gentle swirling in 1 M-KCl (1 ml) for 5 min at room temperature. The mixture was transferred quantitatively to a glass vial with the aid of 9 ml of distilled water and the suspension was mixed gently on a rotator until homogeneous (about 30 min). CsCl (4.3475 g) was dissolved in 3.4 ml of the crude DNA solution and the refractive index of the latter was adjusted to 1.4020 at 25°C by using a Zeiss model A Abbé refractometer. The whole sample was passed three times through a 23-gauge hypodermic needle to shear the DNA (Sinclair, 1974) and was then placed in a Beckman polyallomer centrifuge tube [0.32 cm × 7.62 cm ($\frac{1}{8}$ in × 3 in)]. The solution was overlaid with sufficient white paraffin oil (Liquid Petrolatum) to fill the centrifuge tube, and centrifugation was performed at 20°C and 110000$g_{max.}$ for 66 h in a Beckman type 40 fixed-angle rotor (Flamm *et al.*, 1966) in a Beckman L2-65B preparative ultracentrifuge. The centrifuge was allowed to stop without the brake, the tubes were pierced and 7-drop fractions were collected by using a fraction collector and a peristaltic pump. The density of every fourth fraction was determined from its refractive index at 25°C (Sober, 1970), and the acid-insoluble radioactivity of each fraction was measured by the method of Furano (1971). A 24 cm diam. Whatman no. 3 filter paper, pre-soaked in 5% (w/v) trichloroacetic acid and dried, was marked in numbered rectangles (3 cm × 4 cm). Samples (150 μl) of the fractions were applied to the corresponding rectangles. The filter paper was air-dried and washed in a Buchner funnel with 3 × 300 ml of 5% (w/v) trichloroacetic acid and 2 × 100 ml of ether, with the use of filter-pump suction to remove the last drops of each wash fluid only. The paper was air-dried and cut into the rectangles whose radioactivity was counted as described below, except that a Searle Isocap 300 liquid-scintillation counter was used to obtain the ^{3}H and ^{14}C c.p.m. directly.

Radioactivity assay. Radioactive samples, either liquid or strips of t.l.c. plastic-backed sheets, were counted in 10 ml of PCS/xylene (2:1, v/v). A Beckman LS-230 liquid-scintillation counter was used and the approximate counting efficiency for ^{3}H was 40–45% in solution and 25–30% on the t.l.c. sheets. In the latter case, it was important that the strips be no larger than 2.5 cm × 0.7 cm or there was a decrease in counting efficiency.

Results and Discussion

The survival curves for *H. cutirubrum* irradiated with u.v. light in either the synthetic medium of Grey

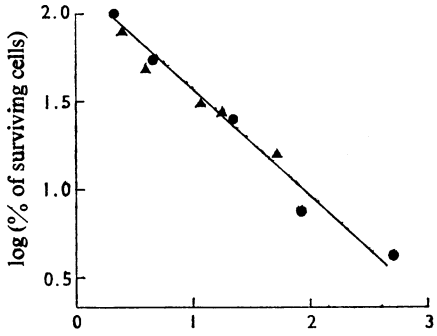

Fig. 1. *Survival curves for H. cutirubrum irradiated with u.v. light*

H. cutirubrum growing in either the synthetic (●) or the complex (▲) medium was irradiated with 11×10^{-7} J/mm² per s (11 erg/mm² per s), and the number of surviving viable cells determined as described in the Experimental section.

Table 1. *Photoreactivation and the absence of dark-repair in u.v.-irradiated H. cutirubrum*

Irradiation with u.v. light [4.5×10^{-4} J/mm² (4500erg/mm²)] and photoreactivation were performed as described in the Experimental section. Viable cell counts were determined on the synthetic medium (Grey & Fitt, 1976) supplemented with 2% (w/v) agar. To test for dark-repair, the Petri dish containing irradiated cells was covered with aluminium foil, placed on ice and swirled exactly as described for photoreactivation, but without illumination. The original control culture contained 2×10^9 cells/ml.

	No. of viable cells/ml	
Time (min)	Visible light	Dark
0	4×10^5	2×10^5
10	6×10^6	3×10^5
50	1.8×10^9	2×10^5

& Fitt (1976) or Gochnauer & Kushner's (1969) complex medium are shown in Fig. 1. The growth medium did not affect the sensitivity of the bacteria, which was about the same as observed by Hescox & Carlberg (1972) in another complex medium, so all other experiments in this paper were done in the synthetic medium. The viable counts shown in Fig. 1 were determined on the complex medium containing 2% agar; the same results were obtained by using the synthetic medium plus agar, so the nature of the solid medium does not affect the colony-forming ability of the irradiated cells.

In Table 1, the viability of samples of a culture irradiated with 4.5×10^{-4} J/mm² (4500erg/mm²) of u.v. light and then either photoreactivated or kept in the dark is compared with that of an untreated control sample of the same culture. Photoreactivation for 50min restored the original number of viable cells, whereas no restoration of viability occurred during the same time in the dark.

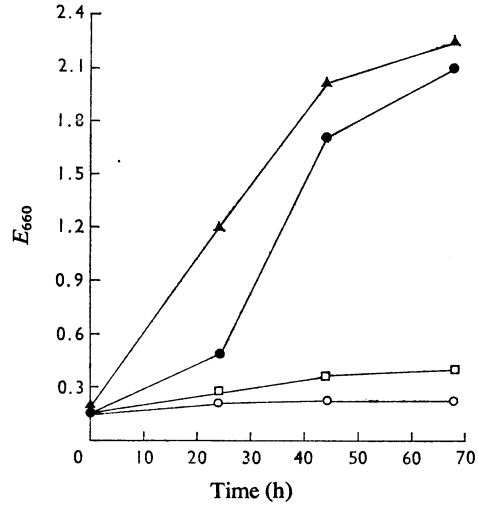

Fig. 2. *Effect of u.v. irradiation with and without photoreactivation on the growth of H. cutirubrum*

Portions (15ml) of a culture of *H. cutirubrum* growing in the synthetic medium were irradiated with 1.8×10^{-4} J/mm² (1800erg/mm²) or 4.5×10^{-4} J/mm² (4500erg/mm²). A culture subjected to the higher dose was photoreactivated for 50min. Samples (5ml) of the treated cultures and of the original untreated control culture were then transferred to flasks containing fresh synthetic medium (50ml) previously warmed to 37°C. The flasks containing the non-photoreactivated samples were covered with aluminium foil. Incubation was continued at 37°C in the standard conditions and growth was determined by turbidimetry at 660nm. Control culture, ▲; culture irradiated with 4.5×10^{-4} J/mm² and photoreactivated, ●; cultures irradiated with 1.8×10^{-4} J/mm², □, and 4.5×10^{-4} J/mm², ○, and kept in the dark.

Fig. 2 compares the growth over an extended period of u.v.-irradiated cultures of *H. cutirubrum* that had been photoreactivated or kept in the dark with that of a control culture. The culture that was irradiated with 4.5×10^{-4} J/mm² and photoreactivated for 50min grew as well as the control, after a lag phase which was probably caused by the need to cool the culture on ice during photoreactivation to prevent damage from the heat of the fluorescent lamp. In contrast, the cultures kept in the dark after irradiation with either 1.8×10^{-4} J/mm² (1800erg/mm²) or 4.5×10^{-4} J/mm² (4500erg/mm²) showed little or no growth during the same period.

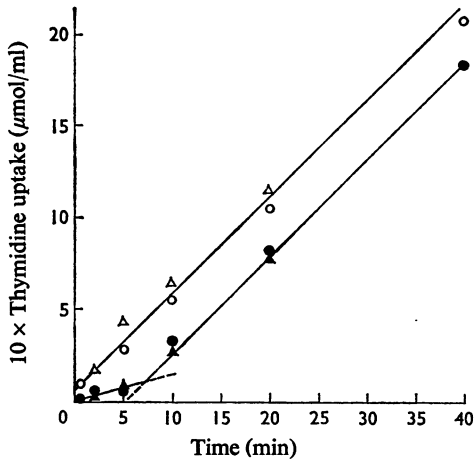

Fig. 3. *Incorporation of ^{3}H-labelled thymidine into DNA by H. cutirubrum*

H. cutirubrum was grown at 37°C in the synthetic medium supplemented with 82.6 μM-thymidine until the cell density was approx. 2×10^8 cells/ml (27 h). The bacteria were collected by centrifugation at 30°C and 28000$g_{max.}$ for 5 min and resuspended in the same volume of pre-warmed (37°C) synthetic medium, pH 7.1, containing thymidine (4.2 μM or 82.6 μM). After 30 min at 37°C, [*Me*-^{3}H]thymidine (sp. radioactivity 18 Ci/mmol) was added to a final concentration of 20 μCi/ml. Samples (1 ml) were withdrawn at the indicated times and added to ice-cold 25% (w/v) NaCl/0.02M-KCN (5 ml) containing a 20-fold excess of unlabelled thymidine. The cells were collected by centrifugation at 45000$g_{max.}$ for 5 min, washed once by resuspension in 25% (w/v) NaCl (10 ml) followed by centrifugation and lysed with ice-cold 5% (w/v) trichloroacetic acid (2 ml). A sample (100 μl) of the mixture was counted for radioactivity to determine the total uptake and the remainder was centrifuged. The precipitate was washed twice by resuspension and centrifugation in a total of 5 ml of ice-cold 5% (w/v) trichloroacetic acid and finally resuspended in 1 ml of the same acid. The suspensions were heated at 85–90°C for 40 min (Hutchison & Munro, 1961) and then centrifuged. The radioactivity of 50 μl of each supernatant was counted as described in the Experimental section to determine the incorporation into DNA. Total uptake of radioactivity: with 4.16 μM-thymidine, ○; with 82.6 μM-thymidine, △. Incorporation into DNA: with 4.16 μM-thymidine, ●; with 82.6 μM-thymidine, ▲.

The results in Table 1 and Fig. 2 confirm that *H. cutirubrum* has a highly effective photoreactivating system and prove that there is no restoration of the viability of the u.v.-irradiated cells in the dark. The bacteria are clearly unable to perform dark-repair of their DNA. However, it was conceivable that, despite the lack of DNA reactivation, the non-viable cells could incorporate small precursors into their damaged DNA without complete repair of the latter. To eliminate this possibility, which would vitiate experiments based on the assumption that the incorporation of precursors into DNA in *H. cutirubrum* was associated solely with replication, we studied the rate of incorporation of thymidine into DNA by normal and u.v.-irradiated cultures of the bacterium.

Table 2. *Evidence that H. cutirubrum incorporates [^{3}H]-thymidine into DNA*

H. cutirubrum was grown in the synthetic medium supplemented with 82.6 μM-thymidine. After 40 h at 37°C [^{3}H]thymidine (20 μCi/ml) was added and incubation was continued for 1 h. Samples (1 ml) were withdrawn and added to ice-cold 25% (w/v) NaCl/0.02M-KCN (10 ml). The cells were collected by centrifugation at 45000$g_{max.}$ for 5 min and were lysed by suspension in distilled water (1 ml). A solution of the appropriate degradative enzyme (5.0 mg/ml) in 50 mM-sodium phosphate buffer, pH 6, was added to give final concentrations of 50 μg/ml of the enzyme and 0.5 mM-phosphate, and the mixture was incubated at 37°C for 30 min. DNA was extracted and its radioactivity counted as described in Fig. 3.

Treatment	Radioactivity (%)
None (control)	100
DNAase digestion	6.4
Pronase digestion	98.9
RNAase digestion	98.9

No previous studies of nucleic acid synthesis in extremely halophilic bacteria *in vivo* have been reported, so it was necessary to find conditions that would permit analysis of the incorporation of a suitable precursor into their DNA. The results in Fig. 3 show that *H. cutirubrum* growing at 37°C in the synthetic medium of Grey & Fitt (1976) supplemented with [^{3}H]thymidine incorporated this precursor into an acid-insoluble form. The latter was degraded by pancreatic DNAase and resistant to both Pronase and RNAase and was therefore DNA (Table 2). The pH of the resuspension medium was always adjusted to 7.1 after autoclaving to avoid any shock to the cells, because during growth of *H. cutirubrum* in the synthetic medium the pH of the latter rises from 6.6 to 7.1–7.2. Incorporation of radioactive thymidine into DNA was initially less than its total uptake by the cells, but the rates became identical after about 5 min (Fig. 3). The shortness of this lag phase compared with the 8 h generation time of the organism in these conditions shows that the total intracellular pool of intermediates between thymidine and DNA must be small, thus permitting its rapid equilibration with the extracellular precursor. It can also be seen that a 20-fold variation in the exogenous thymidine concentration had no effect on either the lag phase or the maximum rate of incorporation of radioactivity into DNA. It can be concluded that the system for uptake of thymidine into the cell becomes saturated at very

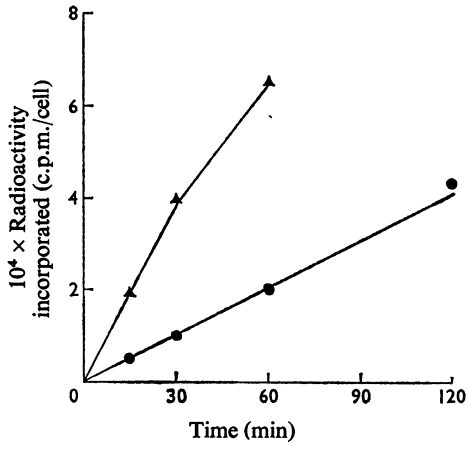

Fig. 4. *Effect of u.v. irradiation on [^{3}H]thymidine incorporation by H. cutirubrum*

The culture and irradiation of *H. cutirubrum* were carried out as described in the Experimental section. The u.v. dose was 1.32×10^{-4}J/mm^2 (1320erg/mm^2). Incorporation of [^{3}H]thymidine (4.16μM, sp. radioactivity 4.8Ci/mol) into DNA was measured as described in Fig. 3. During the incorporation experiment, the flask containing the irradiated cells was covered with aluminium foil. The viable cell counts were 4×10^9 cells/ml in the control culture and 1×10^9 cells/ml in the irradiated culture. Control culture, ▲; irradiated culture, ●.

low concentrations and incorporation of the nucleoside into the precursor pools has little effect on the rate of endogenous DNA precursor synthesis.

The rates of incorporation of thymidine into DNA by unirradiated and u.v.-irradiated *H. cutirubrum* cells were then compared (Fig. 4). In these experiments, cells irradiated with a total of 1.32×10^{-4}J/mm^2 (1320erg/mm^2) were used so as to ensure that some measurable synthesis of DNA would occur in the irradiated, but non-photoreactivated, culture. It should be noted that the results are expressed in terms of the incorporation observed per viable cell, and not per ml of culture or number of original cells in the sample, so as to avoid the artifact of apparent inhibition of DNA synthesis in irradiated cultures caused (Smith & O'Leary, 1968) by the decrease in the number of viable cells. The results in Fig. 4 show that the rate of incorporation per viable cell in the irradiated culture was less than in the control, the maximum values being 3.9×10^{-6}c.p.m./min and 12×10^{-6}c.p.m./min respectively. Thus irradiation caused an actual inhibition of DNA synthesis in the surviving cells and there was no evidence of the large increase in the rate of thymidine incorporation that would be expected if excision-repair of u.v.-damaged DNA occurred in the non-viable cells (Huang *et al.*, 1972).

Table 3. *Release of acid-soluble thymidine and thymidine derivatives by u.v.-irradiated H. cutirubrum*

H. cutirubrum was grown at 37°C in the synthetic medium (20ml) supplemented with 82.6μM-thymidine to a cell density of 7×10^9 cells/ml. The internal pools were then labelled with [^{3}H]thymidine (20μCi/ml) for 30min at 37°C. The cells were collected by centrifugation and resuspended in fresh synthetic medium (20ml) containing unlabelled thymidine (4.16μM). Portions (5ml) of the culture were irradiated with 1.44×10^{-4}J/mm^2 (1440erg/mm^2) (Expt. 1) and 3.6×10^{-4}J/mm^2 (3600erg/mm^2) (Expt. 2) of u.v. light respectively and the remainder was the non-irradiated control. All three cultures were shaken (250rev./min) in the dark at 0°C on a small gyratory shaker. Samples (1ml) were withdrawn at the indicated times and added to 10ml of 25% (w/v) NaCl/0.02M-KCN. The supernatant obtained after centrifugation at 45000$g_{max.}$ for 5min was counted to measure the release of radioactivity into the extracellular fluid. The cell pellet was lysed in ice-cold 5% (w/v) trichloroacetic acid (3ml) and the radioactivity of the acid-soluble and acid-insoluble fractions was determined. The results show the distribution of radioactivity in the 1ml samples.

	10^{-4} × Radioactivity (c.p.m./ml)			
Time (min)	Extracellular	Acid-soluble	Acid-insoluble	Total
Control				
0	3.7	58.2	31.5	93.4
10	4.4	40.0	32.1	76.6
25	3.5	50.4	22.9	76.8
Expt. 1				
0	4.4	46.6	25.1	76.1
10	3.9	39.7	30.8	74.4
25	4.8	51.4	35.1	91.3
40	4.1	53.9	33.8	91.8
Expt. 2				
0	4.6	43.8	34.3	82.7
10	5.0	60.9	28.0	93.9
25	4.8	51.2	26.3	82.3

The absence of any stimulation of thymidine incorporation was not due to a decrease in the specific radioactivity of the thymidine caused by release of unlabelled thymidine or its readily dephosphorylated nucleotides from the non-viable cells for the following reasons. First, incorporation in the irradiated culture was linear throughout the experiment; release of intracellular material would therefore have to be instantaneous, despite the fact that cells irradiated with a u.v. dose of 1.32×10^{-4}J/mm^2 (1320erg/mm^2) can be photoreactivated completely and do not distintegrate rapidly. Secondly, the intracellular pools concerned are small as shown above. Thirdly, the results in Table 3 show that irradiated cells (kept at 0°C to prevent DNA synthesis or possible excision of thymidine dimers) do not release significantly more acid-soluble thymidine or thymidine derivatives from prelabelled pools than unirradiated control cells, so that

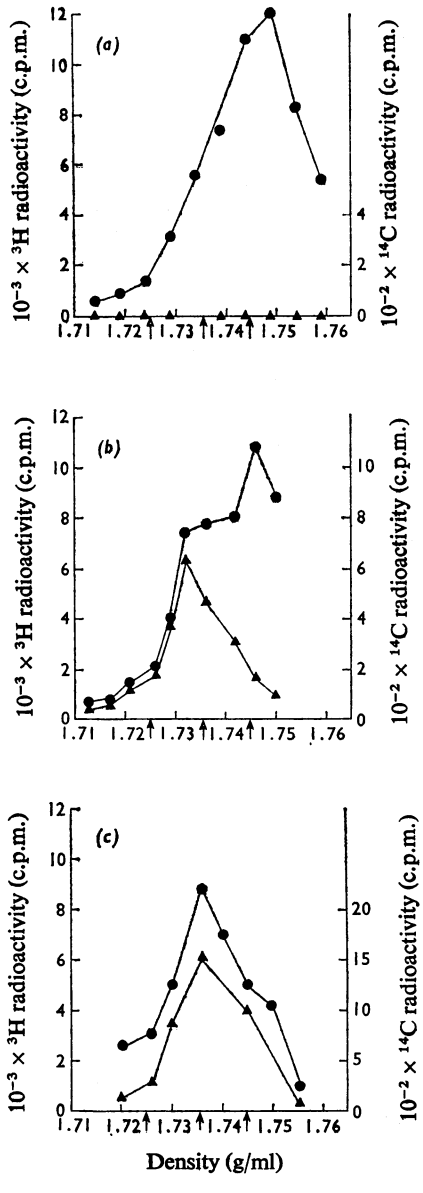

Fig. 5. *Evidence for semiconservative replication and the lack of dark-repair of DNA during normal growth of H. cutirubrum*

The experiment was done as described in the Experimental section. The results show the distribution of 5-bromo-2′-[^{3}H]deoxyuridine (●) and [^{14}C]thymidine (▲) after transfer of the cells to the 'light' thymidine-containing medium: (*a*) zero time; (*b*) 0.125 generation (1 h); (*c*) 1 generation (8 h). The arrows indicate the buoyant densities of the 'light' (1.725 g/ml), hybrid (1.736 g/ml) and 'heavy' (1.748 g/ml) DNA molecules. In each case, 22 fractions were collected in the density range 1.69–1.77 g/ml.

irradiation causes no sudden change in their permeability to such substances during the time-span of these experiments.

Finally, we studied the possibility that some significant amount of repair biosynthesis might occur during normal growth of unirradiated *H. cutirubrum*. The results in Fig. 5 were obtained by using a modification of Meselson & Stahl's (1958) demonstration of semiconservative replication in *Escherichia coli*. Hanawalt & Cooper (1971) have shown that a radioactive precursor incorporated into DNA at early times during a density-labelling experiment is associated with the parental instead of the hybrid daughter molecules if its incorporation is due to repair biosynthesis. In our experiment, the cells were grown first in the presence of the heavy 5-bromo-2′-[^{3}H]-deoxyuridine for several generations and then transferred to fresh medium containing the light [^{14}C]-thymidine, so as to ensure measurable changes in density, because *H. cutirubrum* N.R.C. 34001 incorporates the heavy analogue very slowly, and its DNA (Moore & McCarthy, 1969) has a low percentage of A+T. The results in Fig. 5 show that normal semiconservative replication occurs in *H. cutirubrum* because all the DNA was in the hybrid form after one generation (8 h). Further, even after only 0.125 of a generation (1 h) after transfer to 'light' medium almost all the newly incorporated [^{14}C]thymidine was found at the hybrid density and very little was associated with the heavy parental DNA. Thus no significant repair biosynthesis of DNA occurs during normal replication of unirradiated *H. cutirubrum*.

In conclusion, the results presented above show that wild-type *H. cutirubrum* does not perform dark-repair of DNA, either after irradiation with u.v. light (Figs. 2 and 3 and Table 1) or during normal growth (Fig. 5). It appears to rely on an efficient photoreactivating system for repair of its DNA; in its natural habitat it is exposed to intense sunlight and selection may have occurred for a cell able to use the energy of the incident light to repair damage caused by the latter. These properties suggest that *H. cutirubrum* may have some unique advantages for the study of both DNA replication and photoreactivation.

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