presence of vitamin K_1 in heart sarcosomes after the injection of radioactive vitamin K_3. The observations of MARTIUS AND NITZ-LITZOW[10] suggesting a role of vitamin K_1 in oxidative phosphorylation must also be kept in mind in this connection. The work is continuing, and will later be published in greater detail.

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An extract promoting the synthesis of deoxyribonucleic acid in U.V.-irradiated *Salmonella typhimurium*

A very striking effect of radiation on some micro-organisms is the alteration of deoxyribonucleic acid (DNA) synthesis[1,2,3]. KANAZIR AND ERRERA[2] have established that DNA synthesis is temporarily inhibited immediately after irradiation in *E. coli*, while the growth rate and the synthesis of ribonucleic acid (RNA) and protein are not fundamentally altered. This inhibition of DNA synthesis is followed by an accumulation of DNA precursors and adenosinetriphosphate (ATP), suggesting that a relationship exists between the synthesis of DNA and the metabolism of ATP[4]. This is also true in irradiated *Salmonella*[5].

The aim of this work was to look for factors able to promote DNA synthesis during the period of its immediate inhibition, subsequent to U.V. irradiation, and to elucidate the reasons for the continual occurrence of death as a result of irradiation, in spite of a resumed synthesis of DNA.

Experiments were carried out on *Salmonella typhimurium*, strain LT2, nonlysogenic, grown on aerated synthetic media, whose composition has already been described[5]. The bacteria were irradiated in logarithmic phase, with a Mineralight low-pressure mercury vapour lamp, from a distance of 30 cm, using a dose that gave about 50% survivors. All incubations were done in the dark. The determinations of the acid-soluble fraction, DNA, RNA and protein were carried out as already described[5].

An active principle was extracted from a normal strain of *Salmonella* grown on an aerated broth medium, by grinding fresh cells with 0.15 *M* KCl at 0°. The supernatant was recentrifuged at 0° C and 16,000 r.p.m., and dialysed against phosphate buffer pH 7.0. The crude extract was fractionated with solid ammonium sulphate. The precipitates obtained at different degrees of saturation were dissolved in potassium phosphate buffer at pH 7.4 and dialyzed against the same buffer.

In all tests, irradiated bacteria and controls were grown on synthetic aerated media in the presence of this extract or its ammonium sulphate fractions. An identical amount of protein derived from this extract, 30 γ/ml of culture, was used per assay. The activity of the extract and its fractions was destroyed either by heating or by trypsin digestion, while DNase, and RNase did not cause any inactivation. The extract was added either immediately after irradiation, or after the irradiated cultures had been allowed to grow for 120 minutes. In both cases, the cultures were grown for 20 min after the addition of the extract.

If, after irradiation, the bacteria are grown aerobically in the presence of the crude extract and then plated, the number of survivors increases: it is two or three times higher than in irradiated untreated cultures (Table I). The number of viable cells in the treated cultures is approximately as high as in normal cultures grown for the same length of time (Table I). This table also shows that the synthesis of DNA does not occur in irradiated untreated cells over a period of 20 min growth, while in the presence of our factor this synthesis follows a pattern similar to that of the normal cells and is accompanied by a more extensive utilization of acid-

TABLE I

THE EFFECT OF CRUDE EXTRACT ON NORMAL AND IRRADIATED CELLS WITH RESPECT TO THEIR DIVISION RATE AND DNA SYNTHESIS

Time (minutes)	Control		Control + crude extract		Control + heated crude extract		Irradiated		Irradiated + crude extract		Irradiated + heated crude extract	
	N.A.*	M.A.*	N.A.	M.A.	N.A.	M.A.	N.A.	M.A.	N.A.	M.A.	N.A.	M.A.
	142	118					75	63				
0	140	120					72	58				
	135	115					70	60				
						Number of colonies per plate**						
	161	148	200	185	160	120	48	49	142	130	55	60
20	160	142	197	179	162	123	50	50	148	132	60	56
	158	144	202	182	165	119	53	46	132	135	65	59
							DNA content γ/culture (Ceriotti)					
0	132.4						130					
20	140.2		206.0		126.8		124.4		148		116.8	
							Acid-soluble fraction. Extinction of U.V. 260 mμ Absorbing precursors/30 ml culture					
0	0.260						0.240					
20	0.370		0.300		—		0.335		0.250		—	

* *N.A.* = nutrient agar, *M.A.* = minimal agar (containing only inorganic salts and the glucose used as an energy source).

** Each column records counts of 3 plates. (Bacterial dilution 10^{-6}).

soluble material absorbing in U.V. light. Furthermore, this promoted synthesis of DNA is followed by an increase in the number of dividing viable cells.

The fractions obtained with 0.2 to 0.5% saturations of ammonium sulphate were slightly active, while those obtained with higher concentrations were more active.

If bacteria were left to grow for 120 min after irradiation and the crude extract then added, the synthesis of DNA was faster than in irradiated, untreated cultures; there was a parallel increase in the cell division rate in the treated cultures, while the number of dividing, viable cells continuously decreased in the irradiated untreated cultures, in spite of their resumed DNA synthesis.

The facts we have presented indicate strongly that our factors can restore and promote, in irradiated cells, the synthesis of genetically "good" DNA, thus bringing about an important recovery from irradiation damage.

The detailed data will be published in the *Bulletin of the Institute of Nuclear Sciences "Boris Kidrich"*.

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