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Respiration of Gamma Irradiated *Brucella abortus* and *Mycobacterium tuberculosis*.* (27917)

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Although extensive investigations of the oxidative metabolism of both *Brucella abortus*(1-4) and of *Mycobacterium tuberculosis* (5-6) on various substrates have been carried out, only limited studies of the effect of gamma irradiation on their metabolic activity have been published(7). The preliminary observations demonstrated that bacterial cells may be irradiated with gamma rays in such a manner that they neither multiply on appropriate culture media nor produce disease in experimental animals, yet continue to consume oxygen in the presence of suitable substrates. Herewith are reported additional oxygen uptake studies on gamma irradiated *Br. abortus* and on *M. tuberculosis*.

Method. A gamma irradiated vaccine – G.I.V. – was prepared from a commercially lyophilized and vacuum sealed *Br. abortus* 19.† Some of the vials were equilibrated with oxygen or nitrogen or with air prior to irradiation. For comparative studies heat and chemically inactivated *Br. abortus* vaccines were prepared from 48-hour cultures on Albimi agar. Heat inactivation was carried out in a water bath at 60°C for 10 minutes, and the 5 chemically inactivated vaccines were prepared by 72-hour exposure at 5°C to 50% ether, 5% formalin, 2% phenol, 50% alcohol, and 50% acetone, respectively. In the case of the vaccine from *M. tuberculosis* H37RV, the cells were cultured on Proskauer-Beck medium for one month, washed and resuspended in 5% Dubos albumin for lyophilization and irradiation.

Irradiation of the lyophilized cells was carried out by exposure to a cobalt-60 source, ranging from 2×10^4, 10^5, 2.5×10^5, 5×10^5, and 10^6 r. All vaccines were tested for inactivation by culture and by guinea pig inoculation.

Manometric measurements of oxygen uptake were fashioned after the standard method of Umbreit(8). The procedure of Cameron and Meyers(1-4) was followed closely for the *Brucella* respiration studies, and with slight modification in the case of *M. tuberculosis*. A 20 mg per ml suspension of the tubercle bacillus was employed instead of the 0.7 mg of cellular nitrogen recommended for the *Brucella*. One per cent solutions of D-alanine, L-asparagine, L-glutamic acid, and glucose were used as substrates for *Brucella*. Five-tenths molar solutions of acetic acid, glycerin, lactic acid, and pyruvic acid, the substrates reported to be most vigorously metabolized by *M. tuberculosis* were used(1-5). Both endogenous and exogenous oxygen uptake rates were determined, the latter in duplicate.

Results. Cells of *Br. abortus* and *M. tu-*

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TABLE I. Oxygen Uptake of Lyophilized Gamma Irradiated *Br. abortus* 19 (Q O_2 per mg N).

Atmosphere	Substrate (10 mg/ml)	Dosage of irradiation: 0	2×10^4r	10^5r	5×10^5r	8×10^5r	10^6r
Vac.	D-alanine	132	119	113	87	75	70
	L-asparagine	162	159	154	94	83	78
	L-glutamic acid	340	304	270	188	157	136
	Glucose	186	176	150	108	103	87
N_2	D-alanine		118	115	86	75	
	L-asparagine		157	148	92	81	
	L-glutamic acid		300	267	190	155	
	Glucose		180	150	110	99	
Air	D-alanine		98	95	77	71	
	L-asparagine		148	120	77	76	
	L-glutamic acid		275	233	155	142	
	Glucose		145	131	100	89	
O_2	D-alanine		96	89	67	66	
	L-asparagine		145	118	77	75	
	L-glutamic acid		271	223	142	135	
	Glucose		133	117	90	87	

berculosis exposed to 7.5×10^5 r or greater radiation failed to grow on appropriate culture media. A few colonies, however, were isolated in culture from approximately 10^9 cells irradiated at 5×10^5 r.

Non-irradiated *Br. abortus* oxidized glutamic acid at the highest rate, and this system was slightly more sensitive to irradiation than that with the other substrates (Table I). Irradiation under nitrogen had no advantage over irradiation in the vacuum state, but irradiation under air and under oxygen resulted in similar and slightly accelerated decreases in oxygen uptake with increasing doses of irradiation. The heat and chemically inactivated cells of *Br. abortus* showed no evidence of oxidation except in D-alanine in which the ether and formalin treated cells exhibited slight activity (Table II).

The non-irradiated *M. tuberculosis* respired only in the range of 0.5 to 1 ml of O_2/mg of cells (Table III). Lactic acid was most actively oxidized and the enzymes involved in this reaction were least sensitive to irradiation, while those inherent in oxidation of glycerin were the most sensitive.

Discussion. The present study indicates that the factors concerned with the reproductive function of bacterial cells are more sensitive to irradiation than their respiratory enzymes. Results of extensive studies have been published, and many speculations have been made on the nature of the mechanism of cell inactivation by ionizing radiation(9-12).

TABLE III. Oxygen Uptake of Lyophilized Gamma Irradiated *M. tuberculosis* H37RV. (Q O_2 per mg cells)

Substrate (.4 M)	Irradiation dosage: 0	2.5×10^5r	5×10^5r	7.5×10^5r	10^6r
Acetic acid	.55	.48	.42	.25	.13
Glycerin	.64	.58	.43	.12	.06
Lactic acid	1.00	.80	.64	.58	.43
Pyruvic "	.72	.60	.35	.34	.20

It is well recognized that cellular reaction to irradiation is not a single simple event. A complex of complicated reactions is initiated, and almost any cellular component studied has been shown to be damaged to some extent. Cellular damage may be directly in-

TABLE II. Oxygen Uptake of Heat and Chemically Inactivated *Br. abortus* 19. (Q O_2 per mg N)

Substrate (10 mg/ml)	Ether 50%	Formalin 5%	Phenol 2%	Acetone 50%	Alcohol 50%	Heat
D-alanine	50	10	0	0	0	0
L-asparagine	0	0	0	0	0	0
L-glutamic acid	0	0	0	0	0	0
Glucose	0	0	0	0	0	0

flicted by the radiation or secondarily by the free radicals produced by radiation action on various molecules in the surrounding medium or within the cell itself. A suggestion has been made that the initial lesion may be a breakdown of cellular barriers resulting in release of the cell's own enzymes which attack critical molecules(10,11). At the cellular level Alexander and Bacq(11) recognized 3 immediate biological effects of irradiation: (1) physiological, (2) cell lysis or interphase death, (3) delayed or mitotic death. Although some bacterial cells are killed outright (interphase death) by very low doses of radiation, others require large doses to produce a fatal effect and yet are more susceptible to mitotic death(11,12). The cells of *Brucella* and of *M. tuberculosis* belong to the latter group, and this characteristic of relatively greater sensitivity to mitotic death by irradiation was utilized in our studies to produce a non-proliferating but living vaccine by cobalt-60 treatment of virulent cells. Such cells would be incapable of producing disease in susceptible hosts, yet may be sufficiently like the non-irradiated living cells in antigenicity for producing effective immunity. Such cells are more like the disease-producing organisms than a highly attenuated living strain or those killed by exposure to heat or chemicals.

Among the conditions shown to affect the extent of radiation damage is the amount of oxygen and water present during irradiation. Certain reactions are oxygen dependent, while others are not, and the oxygen dependent reactions reportedly require the presence of a certain amount of water for manifestation(11). In "wet" systems the presence of oxygen is reported to increase the damaging effect of a given dose of irradiation by as much as 300%, whereas in the dry state the increase is much less(11). In our study with lyophilized *Brucella,* the presence of oxygen during irradiation with a given dosage decreased the respiratory activity of the cells from 75% to 90% of that of the cells irradiated under vacuum or nitrogen. Further studies are being carried out in our laboratory to determine whether the presence of oxygen and water during irradiation can be utilized to increase the rate of mitotic death at lower radiation doses without concomitant increase in damage to the respiratory enzymes.

With all the multiplicity of cellular reactions to irradiation, on the basis of dose-response curves, loss of reproductive integrity is postulated as a result of damage to only a very few molecules produced by at most a few ionizing particles per cell(11). These molecules, essential for proliferation, may be enzymes, nucleic acids, or proteins. Extensive studies are being carried out in many laboratories to define the point of attack by radiation in destroying the reproductive function of cells. When more is known concerning this, it may be possible to define the conditions for irradiation of cells to render them non-proliferating with minimal damage to other physiologic activities.

Summary. Lyophilized *Br. abortus* 19 and *M. tuberculosis* H37RV were exposed to various doses of gamma irradiation from a cobalt-60 source and tested for oxygen uptake on suitable substrates. Cells exposed from 750,000 to 800,000 r failed to grow on appropriate culture media, yet continue to respire at a rate from 40% to 50% of that of the non-irradiated cells.

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