processes of the tissues. In connection with the role of spleen in the defense mechanism against infections, the presence of relatively large amounts of the inhibitor in this organ is noteworthy. These aspects are presently under investigation.

Summary. The presence of an inhibitor of the electron transport system of a number of mycobacteria and of lung homogenates of normal mice was found in normal mice and in those infected with *M. tuberculosis* $H_{37}Rv$. This inhibitor was previously found in tubercle bacilli grown *in vivo* and in lung extracts from infected mice. This work shows that the amount of inhibitor is greatly increased in animals infected with $H_{37}Rv$. The significance of these findings is discussed.

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Studies of *Mycobacterium tuberculosis* $H_{37}Rv$ Grown *in vivo:* Utilization of Glucose. (26193)

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Segal and Bloch(1) in their study on biochemical differences of *Mycobacterium tuberculosis* $H_{37}Rv$ grown *in vivo* and *in vitro,* compared the respiration of the 2 kinds of cells. They showed that both *in vitro* ($H_{37}Rv$) and *in vivo* ($LH_{37}Rv$) grown organisms had a similar rate of endogenous respiration but differed in their response to exogenous substrates. While respiratory response to glucose and its intermediates by $H_{37}Rv$ cells ranged from 59 to 102% depending on the substrate used, tubercle bacilli separated from lung tissues gave no response to any of these substrates above that of the endogenous. Discussing the failure of the $LH_{37}Rv$ cells to oxidize glucose and its intermediates, these authors suggested that this may be caused either by lack of permeability of $LH_{37}Rv$ cells to glucose or by an over-supply of endogenous food reserves. Although these factors would not readily explain the specific differential response to salycilic acid and fatty acids, neither of these possibilities was ruled out.

Reports from this laboratory(2,3,4) have shown that tubercle bacilli grown *in vivo* inhibit the electron transport system in cell-free preparations of various mycobacteria and in lung homogenates of normal mice. Inhibition of the electron transport system by tubercle bacilli grown *in vivo* is brought about by an as yet unidentified substance which has its origin in the host. This substance upon adsorption on the surface of the tubercle bacilli confers on them the inhibitory activity. In the light of these findings one can also assume that the inhibitor by coating the *invivo* grown tubercle bacilli interferes in some way with uptake and utilization of glucose by these cells. Since glucose is the only sugar present in animal tissues it seemed highly improbable that *in vivo* grown tubercle bacilli would be incapable of utilizing this important source of energy. We attempted, therefore, to answer this question by studying uptake and utilization of C^{14} Glucose by *in vivo* grown tubercle bacilli.

Materials and methods. The organisms used were: *Mycobacterium tuberculosis* $H_{37}Rv$ and *Mycobacterium tuberculosis* $LH_{37}Rv$. *Mycobacterium tuberculosis* $H_{37}Rv$ was grown and maintained in a liquid medium of the following composition: asparagine, 5.0 g; KH_2PO_4, 5.0 g; citric acid, 1.5 g; ferric ammonium citrate, 0.05 g; $MgSO_4$, 0.5 g; K_2SO_4, 0.05 g; glycerol, 0.5 g; Tween-80, 0.5 g; distilled water, 1,000 ml; bovine albumin to a final concentration of 0.25% was added after sterilization. The tubercle bacilli grown *in vivo* ($LH_{37}Rv$) were obtained from

lungs of moribund mice infected with *M. tuberculosis* $H_{37}Rv$. The infection was caused by intravenous injection into white mice of 15-20 g of 0.2 ml of a 6-day-old culture of $H_{37}Rv$. The cells were separated from the lungs by differential centrifugation described elsewhere(3). For experiments, both the $H_{37}Rv$ and $LH_{37}Rv$ cells after separation from the respective media were washed twice in a 0.1M phosphate buffer, pH 7.2, containing Tween-80 0.05%, and suspended in the same buffer to the desired density as measured in a Coleman Jr. spectrophotometer. A standard curve relating optical density to dry weight of the bacteria was prepared for each strain. Dry weight was obtained by weighing the standard suspensions dried at 105° for 24 hours. In the experiments on uptake of C^{14} glucose, the cells at the end of experiment were centrifuged off, washed and placed on 2 cm^2 aluminum disks, dried, weighed and counted. The counts were corrected to infinite thickness. Oxygen consumption of $H_{37}Rv$ and $LH_{37}Rv$ cells was measured by the conventional Warburg technic. In experiments with C^{14} labeled glucose, the center well contained a 15% solution of CO_2-free KOH, and expired $C^{14}O_2$ was trapped directly into this solution. At the end of experiment, contents of the center well were quantitatively removed into stoppered tubes to which a known amount of carbonate carrier was added. The carbonate was precipitated by dropwise addition with stirring of an excess of 12% $BaCl_2$. The barium carbonate was centrifuged in stoppered tubes and washed several times to remove all traces of alkali. The washed $BaC^{14}O_3$ precipitate was dried *in vacuo,* and the dry powder mounted on disks 0.3 cm^2 and counted at infinite thickness in thin end-window Geiger-Muller tube. Specific radioactivities were obtained by direct comparison with a poly /C^{14}/ methacrylate standard (1 $\mu c/g$) obtained from the Radiochemical Centre, Amersham, Bucks. Standard error in all determinations did not exceed 5%. Uniformly labeled /C^{14}/ glucose was obtained from the Radiochemical Centre, Amersham, Bucks.

Results. Uptake of C^{14} glucose by $H_{37}Rv$ and $LH_{37}Rv$ cells. Our preliminary experiments on respiration of *in vivo* and *in vitro* grown tubercle bacilli showed that both $H_{37}Rv$ and $LH_{37}Rv$ cells had a similar rate of endogenous respiration with Q_{O_2} ranging from 8 to 10. The respiratory response of $H_{37}Rv$ cells to glucose varied from experiment to experiment averaging 30% over that of the endogenous. On the other hand, the response of $LH_{37}Rv$ cells to glucose only occasionally exceeded 5% over the endogenous. These results confirmed the findings of Segal and Bloch(1). To determine whether the low or even complete lack of respiratory response to glucose displayed by $LH_{37}Rv$ cells reflected impermeability of these cells to glucose, we studied the uptake of C^{14} glucose by $LH_{37}Rv$ cells and compared it to that of *in vitro* grown cells. For this purpose, 40 mg of dry weight of $H_{37}Rv$ and $LH_{37}Rv$ cells were added to duplicate 100 ml conical flasks containing C^{14} glucose, phosphate buffer and Tween-80 and the mixture incubated for 30 minutes in a shaker at 37°. At the end of incubation time the cells were removed by centrifugation, washed twice with water and finally measured for radioactivity. The results of a typical experiment are shown in Table I. $LH_{37}Rv$ cells take up C^{14} glucose in considerable amounts, exceeding those taken up by the $H_{37}Rv$ cells. The amount of C^{14} glucose taken up by $LH_{37}Rv$ cells in 30 minutes was 8 μmoles as compared to 5 μmoles taken up by $H_{37}Rv$ cells under identical experimental conditions. These results show clearly that *in vivo* grown tubercle bacilli are readily permeable to glucose and therefore, permeability is not the factor responsible for lack of respiratory response of $LH_{37}Rv$ cells to this substrate.

TABLE I. Uptake of C^{14} Glucose by Non-Proliferating Suspensions of $H_{37}Rv$ and $LH_{37}Rv$.

Cells	C^{14} glucose added		C^{14} glucose taken up	
	μcuries	μmoles	μcuries	μmoles
$H_{37}Rv$	8.0	100.0	.40	5.0
$LH_{37}Rv$	8.0	100.0	.64	8.0

Reaction mixture contained: bacterial cells, 40 mg (dry wt); C^{14} glucose as indicated; 0.1M phosphate buffer pH 7.2, 2.5 ml; Tween-80, 0.05% final conc.; final vol 10 ml; time of incubation 30 min.

Utilization of C^{14} glucose by $H_{37}Rv$ and

TABLE II. Utilization of C^{14} Glucose by $LH_{37}Rv$ and $H_{37}Rv$ Cells.

Cells	Q_{O_2} Endogenous	Q_{O_2} Glucose	Respiration rate, increase over endogenous (%)	Total CO_2 expired (μmoles) Endogenous	Total CO_2 expired (μmoles) Glucose	$C^{14}O_2$ (μmoles)	Radioactivity of $C^{14}O_2$ from total
$H_{37}Rv$	9.11	11.30	24.0	8.48	10.50	2.30	22.0
$LH_{37}Rv$	9.50	9.75	2.6	8.48	8.67	3.21	37.0

Each Warburg vessel contained: bacterial cells, 7.0 mg (dry wt); 100 μmoles of phosphate buffer, pH 7.2; 20 μmoles of C^{14} glucose of total radioactivity 1.66 μcuries; 0.2 ml of CO_2-free KOH, 15% sol in center well; final vol 2.2 ml; time of reaction 3 hr.

LH_{37} Rv cells. After we had shown that glucose was readily taken up by the *in vivo* grown cells, we next performed experiments designed to ascertain whether these cells oxidize this C^{14} glucose. In these experiments we determined respiration rate as well as glucose utilization by both $H_{37}Rv$ and $LH_{37}Rv$ cells. For determination of the extent of glucose oxidation, $H_{37}Rv$ and $LH_{37}Rv$ cells were allowed to respire in presence of C^{14} glucose until approximately 10 μmoles of CO_2 were evolved, then radioactivity content of the expired $C^{14}O_2$ was determined. Results of a typical experiment are illustrated in Table II. The Q_{O_2} of the endogenous respiration of both $H_{37}Rv$ and $LH_{37}Rv$ cells was about 9. Presence of C^{14} glucose in the reaction mixture resulted in an increase of respiration rate of $H_{37}Rv$ cells by 24% over that of the endogenous, while the respiratory response of $LH_{37}Rv$ cells to the added glucose amounted only to 2.6% over the endogenous. Radioactivity measurements of the expired CO_2 revealed that in the case of $H_{37}Rv$ cells, 22% of total CO_2 was radioactive. The radioactivity found in CO_2 expired by $LH_{37}Rv$ cells amounted to 37% of the total. Results of this experiment show unequivocally that *in vivo* grown tubercle bacilli oxidize the exogenous glucose to a greater extent than those grown *in vitro*. Moreover, these results indicate that the lack of respiratory response to glucose by *in vivo* grown tubercle bacilli is apparent but not real, being caused by suppression of their endogenous respiration by glucose. It is apparent that the added C^{14} glucose was without effect on endogenous respiration of $H_{37}Rv$ cells, since the amount of glucose utilized could be wholly accounted for by their increased rate of respiration in its presence.

Discussion. The experiments described here were performed to elucidate the mechanism involved in producing the differences in response to glucose by *in vivo* and *in vitro* grown tubercle bacilli. The experiments on uptake and utilization of C^{14} glucose by *in vivo* grown cells have ruled out both the permeability factor and the inability of *in vivo* grown bacteria to oxidize exogenous glucose as possible causes of the lack of respiratory response to glucose. It was assumed therefore, that other factors might be responsible for this effect; *i.e.*, saturation of the respiratory enzymes involved in glucose oxidation by the endogenous substrates or suppression of endogenous respiration of $LH_{37}Rv$ cells by glucose. One way of distinguishing between these two possibilities would be to determine the respiratory response of $LH_{37}Rv$ cells to glucose at different levels of depletion of the endogenous substrates. This approach is however not feasible since Segal and Bloch (1) showed that endogenous respiration of $LH_{37}Rv$ cells was stable over 20 hour period with no response to glucose during that time. Another approach to this problem would be testing the respiration of cell-free extracts of $LH_{37}Rv$ freed of endogenous substrates by dialysis. Unfortunately, this approach seems also impractical since we have shown(2,3) that $LH_{37}Rv$ cells are coated with a respiratory inhibitor. Disintegration of $LH_{37}Rv$ cells brings this inhibitor in contact with its respiratory enzymes, thus causing a complete abolishment of any electron transfer activity that may have been present in $LH_{37}Rv$ extracts. The results of these experiments also indicated that the inhibitor of the electron transport system, present on the cell surface of *in vivo* grown tubercle bacilli does not in-

terfere with glucose uptake or its utilization by these cells.

Summary. It was shown that *in vivo* grown tubercle bacilli are readily permeable to glucose and oxidize it to an even greater extent than those grown *in vitro*. It was therefore concluded that the apparent lack of respiratory response to glucose by *in vivo* grown tubercle bacilli was not due to either permeability factor or to inability of these cells to oxidize exogenous glucose. Possible causes for the apparent lack of respiratory response of $LH_{37}Rv$ cells to glucose are discussed.

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Cultivation of Two Strains of Killer *Paramecium aurelia* in Axenic Medium.* (26194)

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Certain strains of *Paramecium* contain particles, kappa, in their cytoplasm which are essentially independent genetic units capable of undergoing mutation and self-reproduction. Kappa-bearing paramecia (killers) are capable of killing other particle-free paramecia (sensitives) and are resistant to the action of a toxic agent which they produce(1).

As pointed out by Stock, *et al.*(2), destruction of kappa by chemical means without injury to the host indicates a degree of selectivity on the part of the agent tested. Of over 70 purines and pyrimidines tested by these workers, only 2-6 diaminopurine was found to reduce the kappa content of killers; certain antibiotics have also been reported to have this effect(3). The foregoing experiments were carried out with bacterized cultures. Hence, the role played by these bacteria cannot be adequately evaluated and the need for axenic (bacteria-free) cultures is emphasized.

Reexamination of the kappa system in axenic medium for its possible application to screening potential anti-cancer agents forms the basis of a future investigation. As a preliminary step this paper reports successful development of an axenic medium for 2 killer stocks of *Paramecium aurelia*.

Materials and methods. 1) *Stock cultures.* Killer stocks 51 and 299 of *Paramecium aurelia* and a corresponding sensitive strain of stock 51 were used. Stock 299, a recently discovered killer, contains particles somewhat larger than kappa, called lambda(4). Cultures were obtained from Dr. T. M. Sonneborn of Indiana University, and maintained at 27°C in a 0.15% infusion of Cerophyl (Cerophyl Labs, Kansas City, Mo.) previously inoculated (1-2 days) with *Aerobacter aerogenes;* transfers were made at intervals of 48 hours. In this manner the organisms were maintained at approximately one fission per day and kappa content ranged from 200 to 500 particles per cell. Mass cultures were prepared by doubling the volume with Cerophyl infusion until approximately 1 to 2 l of culture were obtained. Population densities approximated 500-1000 animals per ml. 2) *Sterilization of killer paramecia.* After a number of attempts at sterilization using antibiotics in combination with several washing procedures, the following method gave satisfactory results. Mass cultures of the ciliates (usually 1 or 2 l) were twice filtered through cotton to remove accumulated debris, concen-

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