affects the density of the drops, there is no reason to suspect this, and the results indicate that the salivary glands concentrate deuterium oxide above the level in serum, at least at the low levels of concentration in these experiments. Salivary gland appears to be unique among human tissues so far investigated in this ability.

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Effect of Cell-Free Extracts from *Mycobacterium tuberculosis* $H_{37}Rv$ on Lung Succinooxidase

Segal and Bloch showed that non-proliferating suspensions of human tubercle bacilli grown *in vivo* (*LRv*) exhibited different biochemical properties as compared with the same strain of tubercle bacilli grown *in vitro*: (*a*) they had a lower hydrogen transfer capacity; (*b*) glucose and its intermediates failed to cause an increase over their endogenous respiration[1].

The experiments reported here were concerned with the electron transfer capacity of cell-free extracts from tubercle bacilli grown *in vivo* and *in vitro* and with the effect of these extracts on the respiration of lung homogenates.

The bacilli grown *in vivo* were obtained from the lungs of moribund or dead mice infected intravenously with the human strain of *M. tuberculosis* $H_{37}Rv$. To obtain a good yield each mouse was given intramuscular injections of 1·2 mgm. cortisone acetate every second day starting from the fourteenth day after infection. The bacilli were isolated from the lungs by the method employed by Segal and Bloch[1]. The *in vitro* grown tubercle bacilli strains $H_{37}Rv$ and *BCG* were obtained from 10–20 days cultures on 'Tween' albumin medium. The cells were separated from the culture media by centrifugation and washed twice in 0·1 *M* phosphate buffer *p*H 7·1. Cell-free extracts were obtained by disrupting of the cells in a 9 *KC* Raytheon sonic oscillator for 30 min. and the debris removed by centrifugation at 9,000 r.p.m. for 10 min. at 5° C.

The hydrogen transfer capacity of the extracts was examined by the reduction of triphenyltetrazolium chloride in the presence of different substrates. The cell-free extracts of *BCG* and $H_{37}Rv$ reduce tetrazolium in the presence of lactate, malate and succinate whereas the cell free extracts of $H_{37}Rv$ grown *in vivo* ($LH_{37}Rv$) did not show any activity in this respect. Since it was difficult to believe that $LH_{37}Rv$ extracts would be entirely devoid of hydrogen transfer capacity, the assumption was tested that their inactivity was due to the presence of an inhibitor. $LH_{37}Rv$ extracts were incubated with active *BCG* preparations in the presence of lactate. As seen from the experiment summarized in Table 1, this assumption proved to be correct. The cell-free extracts from $LH_{37}Rv$ inhibited the lactic dehydrogenase of *BCG* extracts from 50 up to 100 per cent.

Table 1. Inhibition of Lactic Dehydrogenase of *BCG* Cell-Free Extracts by Cell-Free Extracts of $LH_{37}Rv$

	O.D. of formazan formed in the presence of lactate by the extracts from			inhibition (per cent)
	$LH_{37}Rv$	*BCG*	*BCG* + $LH_{37}Rv$	
Exp. 1 ..	0·00	2·44	1·22	50
Exp. 2 ..	0·00	3·20	0·00	100
Exp. 3 ..	0·00	2·20	0·24	89

System: Cell-free extracts (equivalent to 6·5 mgm. protein) 0·5 ml. in 0·1*M* phosphate buffer *p*H 7·1; lactate, 0·3 *M*, 0·3 ml.; 1 per cent solution of triphenyltetrazolium chloride, 0·2 ml. Time of incubation 1 hr. Temperature, 37° C. The formazan was extracted with *iso*-butanol and read at 485 mμ in a Coleman Jr. spectrophotometer.

This result prompted us to test the action of $LH_{37}Rv$ extracts on normal lung tissue homogenates. For this purpose lungs of normal mice were homogenized in 0·25*M* sucrose and their oxygen uptake was measured by the conventional Warburg method in the presence of the extracts. Table 2 shows that the extracts of

Table 2. Effect of Mycobacterial Extracts on Succinooxidase of Normal Mice Lung Homogenates

Source of enzyme	μl oxygen per hour*			
	Exp. 1	Exp. 2	Exp. 3	Exp. 4
Lung homogenate	27·2	50·4	33·0	36·1
BCG + Lung homogenate	27·2	53·2	33·0	not examined
$H_{37}Rv$ + Lung homogenate	27·3	43·2	34·3	not examined
$LH_{37}Rv$ + Lung homogenate	15·2	31·1	15·5	20·0

The Warburg vessel contained: 10 per cent suspension of lung homogenate in 0·25 *M* sucrose, 0·4 ml.; where present, bacterial extracts, 0·5 ml.; 0·3 *M* succinate, 0·3 ml. tipped from side arm after 15 min. equilibration; phosphate buffer 0·1 *M*, 0·5 ml. (*p*H 7·1); 15 per cent solution of potassium hydroxide, 0·2 ml. in centre well. The final volume 2·2 ml. Temperature 37° C.
* The values are corrected for oxygen uptake off bacterial extracts.

$LH_{37}Rv$ inhibited the succinooxidase of lung tissue from 31·1 up to 53 per cent. The extracts of $H_{37}Rv$ and *BCG* were without any effect except for one experiment in which $H_{37}Rv$ extract inhibited the oxidation of succinate by 14 per cent.

A detailed report will be given later.

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Seasonal Changes in the Œstrous Response by the Ovariectomized Ewe to Progesterone and Œstrogen

Robinson *et al.*[1] have presented results of the quantitative requirements of progesterone and œstrogen for œstrous behaviour in the spayed Merino crossbred ewe. These results were derived mainly from experiments conducted over short periods (2–3 months) and within the normal œstrous season of that ewe. We have noted[2] that the œstrous response of the spayed Romney ewe following progesterone-œstrogen treatment during November and December (months within the anœstrous season) was less than during the œstrous season. This difference could have resulted from the vasectomized rams exhibiting reduced libido during the summer and so failing to mark all ewes in œstrous[3]. However, our observations suggest that rams in this district will detect ewes in œstrous as we have seen following treatment of ewes