RESPIRATION OF TYPHUS RICKETTSIAE*

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Studies yielding information concerning the growth or metabolism of obligate intracellular parasites should be of obvious theoretical and possibly practical importance. Rickettsiae, a group of intracellular parasites which appear to be physiological as well as morphological intermediates between the viruses and bacteria, seem to offer a favorable group for such studies. The experiments reported below indicate that this indeed may be the case.

Methods

Three strains of typhus rickettsiae were used in this study: the Breinl strain of epidemic typhus, the Madrid E strain of epidemic typhus (3, 5) and the Wilmington strain of murine typhus. The three strains were maintained by serial passage in embryonated eggs (4).

Preparation of Rickettsial Suspensions.—Suspensions of typhus rickettsiae were prepared from infected yolk sac pools which were homogenized in a Waring blendor with 1 volume of a buffered isotonic salt solution and quickly shell frozen in an alcohol-dry ice mixture. The composition of the salt solution is described below. A portion of the 50 per cent yolk sac suspension was thawed, diluted with 2½ volumes of the same salt solution, and centrifuged at 5000 R.P.M. in an angle centrifuge for 45 minutes. The supernatant was discarded; the precipitate was resuspended to the same volume, treated with 1 gm. of celite (6, 12) for each 6 gm. of yolk sac, and centrifuged at 1000 R.P.M. for 30 minutes to remove cell fragments. The supernatant was again centrifuged at 5000 R.P.M. and the precipitate resuspended to the desired volume, usually equal to one-half that of the original yolk sac. This suspension was centrifuged at 500 R.P.M. for 10 minutes to remove any remaining particles; the supernatant turbid fluid constituted the final suspension which was used for measurements of respiration. It generally contained about 50 per cent of the rickettsiae originally present as estimated by its toxicity for mice. The protein nitrogen content was around 0.5 to 1 mg. N per ml., but varied somewhat with each preparation. All procedures were carried out at 0–5°C. and the suspensions were used immediately after preparation.

The salt solution for washing the rickettsiae consisted of 0.122 M KCl; 0.0074 M NaCl; 0.0041 M KH_2PO_4; and 0.0078 M Na_2HPO_4; pH 7.0. In many instances 0.04 per cent casein hydrolysate or 0.0045 M potassium glutamate was also present, except in the solution used for resuspension of the final precipitate. More recently respiration measurements have

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been carried out at pH 7.5 rather than at pH 7.0 as the oxygen uptake is greater at the higher pH. In such cases the final precipitate was resuspended in a solution of the following composition: 0.126 M KCl; 0.0018 M NaCl; 0.0012 M KH_2PO_4; 0.0106 M Na_2HPO_4; pH 7.5.

Oxygen Consumption Measurements.—These were carried out by the conventional Warburg method at 34.3°C. The reaction mixture consisted of 1.5 ml. of a rickettsial suspension; 0.2 ml. of a solution of 0.012 M $MgCl_2$ and 0.004 M $MnCl_2$; substrate, neutralized with KOH, at the indicated concentration, and salt solution of the indicated pH to bring the total volume in the vessel to 2.4 ml. The center well contained 0.1 ml. of 10 per cent KOH. Readings were taken at intervals for 3 to 4 hours but the rates given in Table I were those observed for the first 2 hours.

Glucose concentration was determined by the method of Nelson (9), and pyruvate as described by Lardy (7). Toxicity of the rickettsial suspensions for white mice was estimated by the intravenous injection of 0.25 ml. of serial threefold dilutions of the rickettsial suspensions using 4 mice for each dilution (2). Deaths were counted after 24 hours and the dilution of the final rickettsial suspension required to kill 50 per cent of the mice was estimated by the method of Reed and Muench (11). The infectivity of the epidemic typhus preparations was estimated in cotton rats in the manner described in a previous report (8).

RESULTS

Table I contains the data of the experiments which show that partially purified rickettsial preparations from yolk sac infected with *R. prowazeki*, strain E, had a definite oxygen consumption with casein hydrolysate as substrate. The product of similar preparations from normal yolk sac had an insignificant oxygen uptake under the same conditions indicating that the observed metabolic activity was a property of the rickettsiae themselves. This conclusion was further confirmed by the observation that the oxygen uptake of rickettsial preparations from various pools of strain E was directly related to the concentration of viable rickettsiae as determined by two accepted assays for rickettsiae, namely toxicity for white mice and immunization end-point in cotton rats. The direct proportionality which exists between the rate of oxygen uptake and toxicity for white mice is shown by the constancy of the ratio between these two values given in columns (*k*) and (*l*) of the table. Furthermore, this same correlation of oxygen uptake with the concentration of viable rickettsiae occurred with the Breinl strain of *R. prowazeki* and the Wilmington strain of *R. mooseri*.

The chief constituent of casein hydrolysate responsible for the observed oxygen uptake is probably glutamic acid since this amino acid brings about an oxygen uptake equal to or, usually, greater than that in the presence of casein hydrolysate (see columns (*e*) and (*f*) in the table). Not all of the amino acids have yet been tried, but to date none has been found other than glutamic acid that leads to an increased oxygen uptake by rickettsiae. Carbon dioxide is also produced from glutamic acid, but the R.Q. of 0.85 indicates that the oxidation is incomplete.

No other substrate has been found that is oxidized as rapidly as glutamic acid. It was surprising to find that not only is there no oxygen uptake with

TABLE I

Oxygen Uptake by Purified Preparations of Typhus Rickettsiae

Rickettsiae		pH	Rate of oxygen uptake					Toxicity for mice*	$k_C = e/j$‡	$k_G = f/j$‡
Strain	Pool No.		No substrate	Casein hydrolysate 0.3 per cent	Glutamate 0.0125 M	Pyruvate 0.004 M	Succinate 0.0125 M	LD_{50}		
(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(j)	(k)	(l)
			microliters O_2/hr./ml. rickettsial suspension							
Madrid E	2958§	7.0		5.5			11.5	13	0.42	
Madrid E	2955	7.0	1.9	10.6		4.2	6.4	20	0.52	
Madrid E	2937	7.0		21.1		5.5	19.1	55	0.38	
Madrid E	2956§	7.0	2.3	24.1		8.9	8.9	50	0.48	
Madrid E	3006	7.0		10.4	10.8			17.2	0.60	0.63
Madrid E	3006	7.4		15.7	20.8					
Breinl	3040G	7.0		45.3	61.8	8.1		100	0.45	0.62
Breinl	3040E	7.0			96.4		20.4	172		0.56
Breinl	3040	7.4			107.0	14.1	18.1			
Breinl	3040, frozen‖	7.4			10.0	5.6	39.2			
Wilmington	3096	7.4			28.0	2.9	3.5	55		0.51
Wilmington	3097	7.4			54.5	5.3	4.6	100		0.54
Wilmington	2676	7.4	0.7		87.7	6.8	6.9	172		0.51
Wilmington	2676, frozen‖	7.4			5.5	0.0	23.1	19		0.29
Normal	1	7.0		0.4		0.0	3.1			
Normal	2	7.4			0.5	0.3	1.4			
Normal	2, frozen	7.4			0.4		1.3			

* The toxicity for mice is expressed as that dilution of the rickettsial suspension used for measurements of oxygen uptake, 0.25 ml. of which will kill 50 per cent of the mice.

‡ The constancy of k_C and k_G, the ratio of the rate of oxygen uptake with casein hydrolysate and glutamate respectively to the dilution required to kill 50 per cent of the mice, is a measure of the proportionality between rickettsial viability and respiratory activity.

§ The 50 per cent immunization end-point in cotton rats for pool 2958 corresponded to a dilution of the original yolk sac pool of $10^{6.5}$, that for pool 2956 to a dilution of $10^{7.5}$, when 0.25 ml. amounts were inoculated into cotton rats.

‖ These were portions of the preceding rickettsial suspensions which had been frozen and thawed.

glucose or lactate, but also no disappearance of glucose either aerobically or anaerobically, with or without addition of adenosine triphosphate, cozymase, hexosediphosphate, magnesium, and manganese. However, a very slow oxygen

uptake, roughly proportional to the rickettsial activity, occurs with pyruvate. This oxygen uptake disappears after about 4 hours, in contrast to that with glutamate, which decreases only 10 to 20 per cent in this time. The significance of the small oxygen uptake with pyruvate was checked by measurements of the disappearance of pyruvate in the presence of normal and infected yolk sac preparations. With the former, there was no change in substrate concentration (0.001 M) in 5 hours; with the latter, in one instance 1.2 micromols, with a second more active preparation, 2.3 micromols disappeared in 5 hours.

Succinate increases the oxygen uptake of rickettsiae, but in this instance there is a small oxygen uptake with similar preparations from normal yolk sac. Furthermore, there was no parallelism between rickettsial toxicity and the rate of oxygen uptake in the presence of succinate. It is possible that live rickettsiae may be impermeable to succinate since on freezing in the absence of protein or other protective substances, the viability of rickettsiae as indicated by their toxicity for mice, and their activity toward glutamate and pyruvate are greatly reduced while their rate of oxygen uptake with succinate is doubled or trebled (see table, pools 3040 and 2676).

It is clear from these results that typhus rickettsiae which have been separated from the greater part of the tissue in which they were grown exhibit definite metabolic activity. This phenomenon is quite different in nature from the changes in metabolism observed in some virus-infected tissues. The latter appear to be due to changes in the tissue metabolism brought about by the presence of the virus rather than to activity of the virus itself (1, 10).

SUMMARY

Partially purified suspensions of typhus rickettsiae have been shown to exhibit metabolic activity as evidenced by consumption of oxygen and production of carbon dioxide in the presence of glutamate. Similar activity at a much lower rate occurs in the presence of pyruvate. The rate of oxygen uptake was directly proportional to the concentration of viable rickettsiae, as estimated by their toxicity for mice. Normal yolk sac suspensions prepared in the same manner showed only a very slight oxygen uptake under the same conditions. Glucose was not metabolized by the rickettsial suspensions.

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