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Lysine Deficiency and Leukocyte Metabolism. (31507)

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In a recent publication(1) studies on the mechanism of host resistance to infectious disease were reported. The altered resistance was developed by placing rats on a lysine deficient diet. The interrelationship between altered diets and bacterial infection has been reviewed by Dubos and Schaedler (2) and Schneider(3). Lysine deficiency and its effect on anthrax infection was selected because of the long recognized(4) decreased resistance of herbivorous animals to this infection(5). The results of our earlier studies (1) demonstrated the reduced ability of the reticuloendothelial (RES) to carry out its phagocytic responsibilities. Additional studies (6) showed that under the conditions of the lysine deficiency which caused the lowered resistance to anthrax, there was a decreased ability to mobilize leukocytes. This paper reports continuing studies on the mechanism of the host resistance to infection. It has been shown(7,8) that there is increased oxygen (O_2) utilization by leukocytes during phagocytosis which can be used as a measure of this cellular activity(9). Therefore, the effect of lysine infection on the oxidative metabolism of leukocytes has been studied and is reported here.

Materials and methods. Female rats of the Sprague-Dawley strain were received as weanlings, 22 days old and between 40-50 g, from the supplier. They were randomly divided into control (CON) and experimental (LYS) groups and placed 2 to a cage with a screened floor. The animals were immediately placed on the appropriate diets. These were the same as those used in the previous studies (1) with a slightly different method of preparation. The lysine deficiency was produced by replacing the casein of the control diet with gluten, a protein deficient in this essential amino acid. The two diets were isocaloric. They were prepared by mixing the dry powder with distilled water to a dough-like consistency, placing in aluminum foil moulds and heating in a 250° oven until set. The food was removed from the oven, cooled, cut into squares approximately $2'' \times 2''$, frozen, and held at 0°F until use. The animals were allowed food and water *ad libitum* and kept on the diet for 28 days before use and were not used after 42 days. The weight for each group after 28 days was 77 ± 7 g LYS, 180 ± 3 g CON. These data compare most favorably with the previous report(1).

Peritoneal exudates were stimulated by injecting a 10% solution of glycogen, intraperitoneally, 12 hours and 4 hours before use. The LYS animals received 9 ml and 5 ml and the CON 15 ml and 5 ml at the respective periods. To collect the leukocytes, the rats were anesthetized with ether, the abdomen opened and the fluid in the peritoneal cavity aspirated with a Pasteur pipette. The peritoneal fluid was transferred to a graduated centrifuge tube containing 0.5 ml heparinized Hanks' solution. The peritoneum was washed once with 5 ml (LYS) and 7 ml (CON) of Hanks' solu-

tion and the washings transferred to the corresponding centrifuge tubes kept in crushed ice. The tubes were centrifuged at 700 rpm (International Equipment Co. Model HN) for 8 minutes. The supernatant was discarded and the pellet resuspended in 2.5 ml cold Hanks' solution. Prior to pooling the cells from each group, the cells from individual animals were examined for bacterial contamination by making smears and staining in the routine way with Wright's stain. The cells from individual rats were pooled in the same 2.5 ml cold Hanks' solution and kept in crushed ice until transferred to the Warburg flasks.

Bacillus subtilis (NB 10-22) was the bacterium used for phagocytosis studies. The cells were maintained in nutrient broth. Prior to use, transfers were made to 3 petri dishes containing 10 ml nutrient agar and incubated at 37°C for 18 hours. The bacteria were transferred by rubbing a sterile, bent glass stirring rod over the surface of the agar and suspending the cells in 1 ml of Hanks' solution. The suspensions were collected in a single, sterile, graduated centrifuge tube and autoclaved at 15 psig. for 20 minutes. The supernatant was discarded, the cells washed once in Hanks' solution and finally suspended in 1.5 ml of sterile Hanks' solution.

Oxygen consumption was measured by the direct method of Warburg as described by Umbreit *et al*(10). The center well of the Warburg flask contained 0.2 ml 15% KOH and a 1 × 2 cm fluted filter paper. The main compartment contained 2.5 ml Krebs-Ringer phosphate buffer(10) and 0.1 ml of the killed *B. subtilis* suspension. 0.5 ml of the leukocyte suspension was placed in the side arm of the flask and tipped in at zero time. After being attached to the manometers, the flasks were gassed with 100% oxygen for 5 minutes, placed in the water bath at 37.5°C and equilibrated for 5 minutes. The contents of the side arm were tipped in and readings taken at 5-minute intervals for 45 minutes.

Carbon dioxide was determined by the direct method of Warburg as described by Umbreit *et al*(10). One flask was used as described above and one had 0.2 ml of the buffer instead of KOH in the center well.

TABLE I. Effect of Lysine Deficiency on Oxygen Utilization.

	a Phagocytosis	b Resting	Ratio a/b	a *vs* b P<
1: LYS	.345*(17)	.250*(17)	1.38	.01
2: CON	.191*(17)	.129*(17)	1.48	.01
3: Ratio, (1)/(2)	1.81	1.94		
4: 1 *vs* 2, P<	.01	.01		

( ) No. of samples contributing to mean.
* $\mu l/10^6$ WBC/30 min.

Leukocyte counts were carried out by the usual standard laboratory techniques(11).

Results and discussion. As noted above, the control (CON) animals had a weight gain similar to that previously reported(1). The experimental (LYS) animals, although they did not grow as much as the CON groups, did gain weight. See also(1). The difference in the physical appearance of the two groups was marked. The LYS were smaller, relatively inactive, and had loss of fur.

From Table I, it is obvious that the LYS leukocytes had a significantly increased Q_{O_2} (ul $O_2/10^6$ WBC/30 min) as compared to to the CON group. This increase, about 80-90% persisted even when the cells were phagocytosing. It is also apparent that phagocytosis increases the Q_{O_2} over the resting value, in both groups equally. This point is in accord with the observations of Karnovsky and Sbarra(12) and may be interpreted to mean that both groups are equally effective in the engulfment of the organisms(9). This lends support to the hypothesis presented previously(1) that the increased susceptibility to infection resulting from lysine deficiency could be the result of decreased ability to destory the organism once they have been engulfed.

To determine a possible cause of the increased Q_{O_2} of the LYS group, respiratory quotients (RQ) were determined. Table II shows that there was no difference between the two groups under the same conditions. However, the RQ did go down during phagocytosis, which might be expected with the in-

TABLE II. Effect of Lysine Deficiency on Respiratory Quotient (RQ).

Lysine deficient		Control	
Resting	Phagocytosis	Resting	Phagocytosis
1.23	1.16	1.25	1.13

creased glycolysis that usually accompanies this function(12). What was surprising was a value for the RQ greater than one. Since leukocytes have a significant hexose monophosphate shunt operating, the increased CO_2 production without the corresponding increase in O_2 consumption might be the result of this activity. The TPNH produced during the steps leading to the decarboxylation of 6-phosphogluconic acid is necessary for synthesis for fatty acids. Also, some of the DPNH produced could be used for the production of lactate from pyruvate during anaerobic glycolysis. Thus, the reduced coenzymes could be reoxidized without utilizing molecular oxygen. Despite the fact that the RQs are greater than one, the important point in relation to this determination is that there was no difference in the metabolic pathways in the two groups of animals as reflected in the similar RQs.

We find it difficult to explain the basis for the increased Q_{O_2} of the LYS group over the CON group. However, it has been shown that the adenine-adenine-guanine (AAG) triplet can code for glutamic acid as well as lysine(13). Furthermore, lysine is known to be a major amino acid of the ribosomes(14). In a period of lysine deficiency, these two factors could be responsible for the production of ineffectual protein. As the cell then labors to produce additional protein, an energy requiring function, its O_2 consumption increases to meet this need.

Summary. Q_{O_2} and RQ of rats on a lysine deficient diet have been studied. The Q_{O_2} of the deficient animals is significantly increased, almost 2 times, over control animals. The RQ, although greater than 1.0 is the same in both groups and decreases equally during phagocytosis in the expected manner. The Q_{O_2} likewise increases equally in both groups during phagocytosis. It is concluded that the ingestion of the bacteria is unaffected by lysine deficiency.

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