STUDIES ON THE INTERACTION BETWEEN PHAGOCYTES AND TUBERCLE BACILLI*

III. Some Metabolic Effects in Guinea Pigs Associated with Infection with Tubercle Bacilli

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As part of a study of the host-parasite relationship in tuberculous infection some of the factors governing the respiration and glycolysis of leucocytes have previously been reported (1). Further, the effect of leucocytes on tubercle bacilli has been studied by allowing the former to phagocytize live or dead C^{14}-labelled tubercle bacilli (2). In the present communication the obverse situation is examined, and effects associated with tuberculous infection on some metabolic activities of host cells and tissues are reported.

Experiments have been carried out which belong to two main categories. First, changes in some metabolic activities (notably respiration and glucose oxidation) of leucocytes, brought about by infection of the intact host with tubercle bacilli, have been determined. For comparison with these observations on leucocytes, changes in some metabolic activities of individual organs (liver and kidney) were measured *in vitro* after infection of the intact host, and observations were also made on whole animals. Second, changes in the metabolism of leucocytes from normal and infected animals *during* phagocytosis of tubercle bacilli were determined.

Materials and Methods

Source of Phagocytes, and Respiration Experiments.—The technique used has been described in detail in a previous paper (1). Guinea pigs were used for all experiments, and suspensions rich in polymorphonuclear leucocytes were obtained from the peritoneal cavity by

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the injection of a solution of caseinate 16 hours prior to the experiment. The amount of cellular protoplasm in this suspension was determined by measuring the total cellular phosphorus (1). The leucocytes were suspended in a Ringer phosphate buffer (pH 7.4) containing 33 per cent guinea pig serum. In every experiment peritoneal exudate cells (PEC)[1] from normal and infected guinea pigs were used. Great care was taken to standardize the procedures to ensure identical treatment of all cell suspensions. This was especially important with respect to the period of time which elapsed from the killing of the animal to the first reading of the Warburg respirometer. It was also important to standardize the density of the cell suspensions. The animals were usually killed at half-hour intervals (normal and vaccinated animals were killed alternately) and the first reading with each suspension was made 1 hour later. To ensure similar cellular densities a rough wet weight determination was made after the centrifugation of the cells. Differential counts were also done as described previously (1).

In experiments using labelled glucose, the medium used was a Ringer phosphate buffer, and the radioactive substrate (5 mg. glucose) was tipped in from the sidearm at the beginning of the observation period—*i.e.*, after equilibration of the flasks in the constant temperature bath. When experiments using labelled glucose were carried out with PEC and heat-killed tubercle bacilli, flasks with two sidearms were used and the labelled substrate and the microorganisms were tipped into the main compartment from the separate sidearms at the same time. Controls were carried out as indicated later.

Strains of Tubercle Bacilli Used for Infection.—The following strains of tubercle bacilli were used: BCG obtained from the Henry Phipps Institute in Philadelphia. (Courtesy of Dr. J. D. Aronson); R1Rv obtained from The Rockefeller Institute for Medical Research, New York. (Courtesy of Dr. René J. Dubos); and Vallée from the Public Health Research Institute of the City of New York, (Courtesy of Dr. Hubert Bloch). BCG and R1Rv are attenuated strains of tubercle bacilli, both causing a limited infection, whereas Vallée is a strain exhibiting high virulence for guinea pigs and mice.

The guinea pigs were infected by intradermal or subcutaneous injection of cultures grown for 8 days in liquid medium containing tween 80 and albumin (3). The guinea pigs infected with BCG and R1Rv continued to gain weight and appeared healthy, while those infected with Vallée lost weight and became sick. 4 to 6 weeks after infection some of the latter animals died of generalized tuberculosis. The leucocytes were obtained from the animals about 3 to 5 weeks after infection.

Preparation of Tissue Slices from Liver and Kidney.—Immediately after the animal had been killed, the liver and kidneys were removed and placed in chilled Ringer phosphate solution. Slices were made using a Stadie-Riggs microtome. There was little difficulty in obtaining liver slices that were comparable. In the case of the kidney, however, it was necessary to standardize the slicing. This was done as follows: each kidney was carefully trimmed free of fat and halved longitudinally. Slices were made on the exposed inner surface of each half kidney. Only the first two slices from each half kidney were used.

Determination of Dry Weight.—The dry weight of the tissue, as a percentage of the wet weight, was obtained by placing suitable weighed fragments in small tared cups, drying in an oven for 24 hours at 110°C., cooling in a desiccator, and weighing again. This was repeated to constant weight.

Oxidation of Glucose by Intact Guinea Pigs.—Normal guinea pigs, and animals infected with the Vallée strain 26 ± 3 days prior to the experiment, were given 10 mg. of C^{14}-labelled glucose (1×10^6 C.P.M./10 mg.) dissolved in 1 ml. of physiological saline, by intraperitoneal injection.

[1] PEC in this paper refers to the leucocytes obtained from the peritoneal exudate described above. Of the cells of such exudates, 79.7 ± 2.9 per cent were polymorphonuclear leucocytes (1).

The animals, which weighed between 300 and 350 gm. were placed in a metabolic train immediately, and their expired CO_2 collected in NaOH solution in gas-washing bottles for hourly periods up to 4 hours. From the Na_2CO_3-NaOH solutions suitable dilutions were prepared for CO_2 determinations according to Van Slyke (4). To measure the respiratory $C^{14}O_2$, $BaCO_3$ was precipitated from 2 aliquots of each Na_2CO_3-NaOH solution by adding a $BaCl_2$-$Ba(OH)_2$ solution (2). The precipitate was washed, plated, and counted as previously described (2). From these measurements, the total CO_2 expired per hour could be determined, and also the proportion of the administered glucose-C^{14} which had been converted to CO_2.

In experiments with uniformly labelled glucose-C^{14} seven normal and seven infected animals and in the case of glucose-1-C^{14} or -6-C^{14}, two normal and two infected animals were studied.

In the experiments with glucose-1-C^{14} and glucose-6-C^{14}, each animal received the two radioisomers with an interval of 48 hours intervening. This time period was shown to be sufficient for the elimination of virtually all the activity injected in the first dose.

Radiochemicals Employed.—Uniformly labelled glucose-C^{14} was obtained from the Nuclear Chemical Co., Chicago. Glucose-1-C^{14} and glucose-6-C^{14} were obtained through the courtesy of Dr. H. Isbell, of the National Bureau of Standards, Washington.

Determination of Radioactivity.—Radioactivity measurements were made as described previously using a gas-flow counter in the proportional range (5). The activity of the glucose samples used in the experiments *in vivo* or *in vitro* was determined by converting the glucose to glucosephenylosazone (6) and plating and counting this substance, after recrystallization (7).

RESULTS

1. Respiration of Leucocytes from Casein Exudates of Normal Guinea Pigs and of Guinea Pigs Infected with Tubercle Bacilli.—The results of experiments in which the respiration of PEC from normal guinea pigs was compared with that of PEC from guinea pigs infected with BCG, R1Rv and Vallée respectively, are represented in Fig. 1. As can be seen, the amount of oxygen consumed per unit of protoplasm is highest with cells from guinea pigs infected with the virulent strain (Vallée) and is lowest with PEC from normal animals. The values for the PEC from BCG- and R1Rv-infected guinea pigs lie between the former two. The differences between normal cells and cells from R1Rv- and Vallée-infected animals are significant, whereas respiration of cells from BCG-infected animals is not significantly higher than respiration of PEC from normal animals. It may be noted that PEC from normal animals have an oxygen uptake of 36.5 ± 1.9 μl. O_2 per 100 μg. cellular phosphorus and hour (1).

Experiments were also done in which air was replaced by 1 per cent oxygen and 99 per cent nitrogen. The rate of oxygen uptake per unit of cellular phosphorus was again greater with the PEC derived from guinea pigs infected with tubercle bacilli. For all these experiments 16-hour caseinate exudates were used. Since polymorphonuclear leucocytes (PMN) have an oxygen uptake rate that is only about one-half of that of monocytes (MN) (1) it was important to determine whether the relative proportions of PMN and MN were the same in peritoneal exudates from normal and from infected animals.

Advantage was taken of the fact that for a given number of cells, MN are about one-third heavier (dry weight) than PMN, and also contain about one-third more cellular phosphorus (1). The ratios of dry weight to cell number, and cellular phosphorus to cell number were determined for the exudates from normal and infected animals and were found not to differ significantly. This fact coupled with the results of differential counting of cells indicated

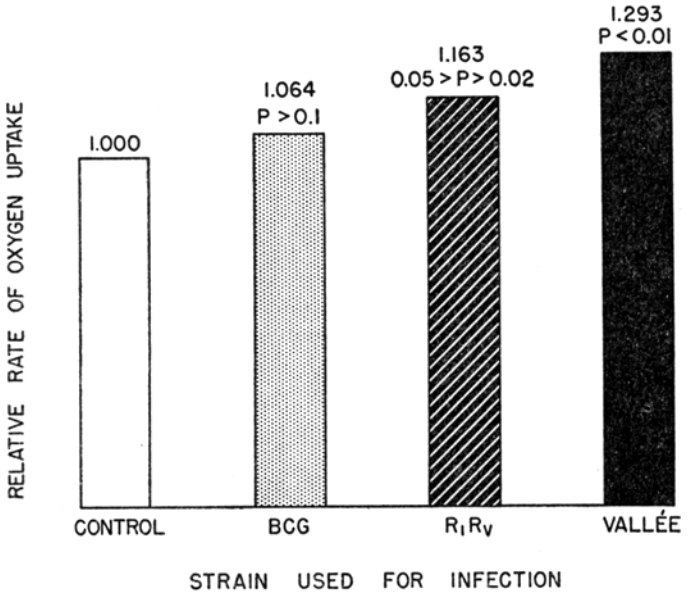

FIG. 1. Relative oxygen uptake rates of leucocyte suspensions rich in PMN from normal guinea pigs and from guinea pigs infected with tubercle bacilli. The oxygen uptake rates were calculated as μl./100 μg. cellular phosphorus and hour. The absolute value for PEC from normal animals is 36.5 $\pm$ 1.9 μl. O_2/100 μg. phosphorus and hour. The number of animals to which each bar refers are as follows: control, 31; BCG-infected, 15; R1Rv-infected, 8; Vallée-infected, 6. With the exudate from each animal, at least two determinations were obtained.

that the cellular compositions of the different exudates did not differ significantly. Viability tests with trypan blue also indicated that there was no difference in the proportion of viable cells in the suspensions from normal and vaccinated guinea pigs. Aerobic glycolysis of leucocytes from infected animals was 3.6 per cent lower than that of leucocytes from normal animals. This difference was not significant. In a further series of 12 experiments using PEC from normal animals and animals infected with the Vallée strain, it was found that the leucocytes from the infected animals had an oxygen uptake 1.57 times that of those from normal animals ($P < 0.01$) thus confirming the results of Fig. 1. In this second series of experiments serum was omitted from

the medium and glucose was added as substrate instead (see paragraph *4* below).

2. Respiration of Liver and Kidney Slices from Normal and Infected Guinea Pigs.—As a result of the above observations, the question arose whether the effects were due to a general stimulation of catabolic processes or whether a mechanism peculiar only to leucocytes was involved. The following experiment was carried out to examine this question.

TABLE I

Respiration of Various Tissue Preparations from Normal and Infected Guinea Pigs

		No. of observations*	Animals		*P*
			Normal	Infected	
Liver	Dry weight‡	10	23.83 ± 0.28	21.90 ± 0.45	<0.01
	Q_{O_2}§	10	3.29 ± 0.18	5.42 ± 0.39	<0.01
Kidney	Dry weight‡	10	20.51 ± 0.58	18.26 ± 0.75	<0.05
	Q_{O_2}§	10	8.03 ± 0.86	9.33 ± 0.99	0.3
Casein exudate cells	Dry weight‖	2	11.05 ± 0.13	14.65 ± 1.1	—
	Q_{O_2} (P)¶	4	21.73 ± 1.32	26.18 ± 0.17	<0.02

* Equal numbers of observations were made on normal and infected animals.

‡ As percentage of fresh weight.

§ Expressed as μl. O_2 per mg. dry weight and hour.

‖ Expressed as mg. per 1×10^8 cells.

¶ Expressed as μl. O_2 per 100 μg. P and hour.

Four male guinea pigs were infected subcutaneously with 0.1 ml. of *Mycobacterium tuberculosis* strain Vallée grown for 8 days in liquid medium containing tween 80 and albumin. Four similar guinea pigs were kept as controls. 3 weeks later the animals were all injected intraperitoneally with caseinate, and on the following day the animals were sacrificed. At one-half hour intervals, the infected and control animals were bled to death alternately. The exudates were collected in the normal way and livers and kidneys were used for the preparation of tissue slices. The results obtained with slices from the liver and kidney and with caseinate-exudate cells are shown in Table I.

Inspection of the figures shows that the difference between the rate of respiration of kidney slices from normal and infected animals is not significant. Liver slices from the infected guinea pig have a significantly higher respiration than those from the controls.

In Table I it may also be noted that the amount of water in the tissue was increased in the infected animals, particularly in the case of the liver. The respiration data have, however, been calculated on the basis of dry weight.

Histological examination of the liver tissue revealed that granulomatous lesions were scattered throughout the liver. There was no necrosis or caseation. One striking feature was the rim of fat at the margin of each lesion. The capsule showed thickening and cellularity due to an acute inflammatory infiltration.

3. Experiments on Intact Animals.—Since leucocytes and liver slices from infected guinea pigs have a higher respiratory rate than those from normal animals, intact animals were examined in an attempt to find whether any differences could be detected in CO_2 production, glucose oxidation or the relative efficiency of elimination of C-1 and C-6 of glucose. The results are given in Table II.

TABLE II

Glucose Oxidation by Normal and Infected Guinea Pigs

Animals	No. of experiments	CO_2 production*	Glucose oxidation‡	Ratio C_1/C_6§
Normal	7	2.86 ± 0.12	(1.94 ± 0.12) × 10^5	1.27 ± 0.04
Infected	7	2.96 ± 0.07	(1.81 ± 0.06) × 10^5	1.35 ± 0.11
P		<0.5	<0.3	>0.5

* Expressed as mM/100 sq. cm. of body surface area and hour.

‡ Expressed as C.P.M. expired in 4 hours for 1 × 10^6 C.P.M. given, and 100 sq. cm. of body surface area.

§ Determinations were made for 2 normal and 2 infected animals. The ratio represents

$$\frac{CO_2 \text{ derived from } C_1 \text{ of glucose}}{CO_2 \text{ derived from } C_6 \text{ of glucose}}.$$

It may be seen that no significant differences were found. It will be noted that the results for glucose oxidation have been expressed on the basis of body surface area, since phenomena of this type have been found to correlate better with surface area than with weight (8). When calculated on a weight basis, *i.e.* counts expired per 100 gm. body weight, there are still no significant differences between normal and infected animals.

If the glucose oxidation is not computed to a standard weight or body surface area, but is expressed only as a percentage of administered counts expired, *i.e.* percentage of administered glucose carbon completely oxidized, there still appear no significant differences between normal and infected animals. The figures are, however, of interest. Thus in 2 hours, normal animals expired 34.5 ± 2.0 per cent, and infected animals 32.0 ± 1.0 per cent of administered glucose carbon. At 4 hours the values were 76.2 ± 3.3 per cent and 74.1 ± 2.6 per cent respectively. The *P* values are greater than 0.2 at 2 hours and greater than 0.5 at 4 hours.

It is also clear from the table that there is no apparent change in the rela-

tive importance of the glycolytic-tricarboxylic acid cycle pathway, and the hexosemonophosphate shunt, as represented by the ratio of expired C-1 and C-6 of glucose (see Fig. 2). The values of Table II refer to the final results at the termination of the experiment. There was no marked difference in the pattern of $C^{14}O_2$ expiration with time between normal and infected animals.

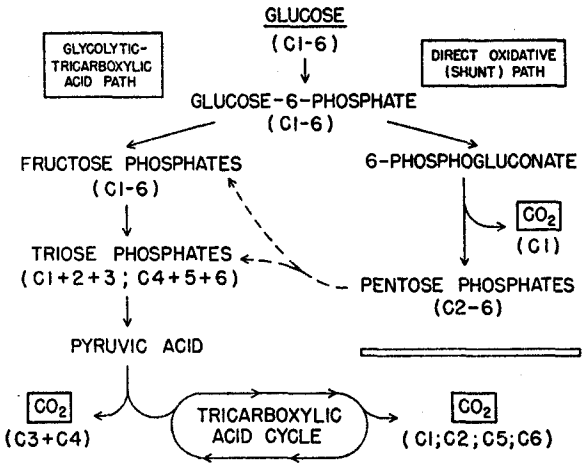

FIG. 2. The conversion of glucose carbon to carbon dioxide via two different pathways. In the case of the pathway on the left, C_1 becomes equivalent to C_6, C_2 to C_5, and C_3 to C_4 at the triose phosphate stage. Thus C_1 and C_6 appear together from the tricarboxylic acid cycle. The C_1/C_6 ratio mentioned in the text would then be 1. Conversion of glucose by the direct oxidative pathway would result in C_1/C_6 ratios much greater than 1, since C_1 would be directly converted to CO_2. The actual ratios observed experimentally reflect the relative quantities of glucose carbon converted to CO_2 by each of these paths. The scheme shown is greatly simplified and several different factors affecting the C_1/C_6 ratio do not emerge. For example, the contribution from the shunt pathway of some fructose phosphate with an arrangement of carbon atoms different from that listed does occur. Further, the reversibility of reactions is not indicated. For a full discussion of these matters, the reader is referred to the review of Horecker and Mehler (22).

4. Oxidation of C^{14}-Labelled Glucose by PEC from Normal and Infected Animals.—Since it had been found (see paragraph *1* above) that leucocytes from Vallée-infected animals had a significantly higher oxygen uptake than those from normal animals, it was of interest to examine the glucose oxidation of these cells. Thus experiments were carried out in which leucocytes were incubated with glucose labelled uniformly (glucose-U-C^{14}) and with glucose-1-C^{14} and glucose-6-C^{14}. Such experiments have been used (9–11) with tissue slices to provide an indication of the magnitude of pathways of origin of CO_2 derived from glucose carbon (see Fig. 2). The results obtained are presented in Table III.

It may be seen that there is no apparent difference between the two groups with respect to the ratios of activity of CO_2 derived from the various types of labelled glucose by PEC. However, the *amount* of radioactive glucose carbon expired as CO_2 by the leucocytes from infected animals was, for all cases, 22 ± 8 per cent higher than that produced by leucocytes from normal animals ($P < 0.02$). Thus the oxidation of glucose carbon confirms the figures for oxygen uptake (see Fig. 1). It should be noted that no serum was added to the medium in these experiments to avoid the addition of lactate or other intermediates which might change the ratio of activity appearing from C_1 or C_6.

TABLE III

$C^{14}O_2$ Evolution by PEC from Glucose-U-C^{14}, Glucose-1-C^{14} and Glucose-6-C^{14}

Group	No. of animals	Glucose labelling			Ratios	
		C_U	C_1	C_6	C_1/C_6	C_1/C_U
		*C.P.M.**	*C.P.M.**	*C.P.M.**		
Normal	4	100 ± 0	229 ± 21	28 ± 4	8.2 ± 1.4	2.3 ± 0.1
Infected	3	125 ± 6	263 ± 21	33 ± 1	8.0 ± 0.7	2.1 ± 0.2

* Expressed as total counts per minute recovered from the center well of the Warburg flask at the termination of the experiment. Results were calculated on the basis of 1×10^5 C.P.M. used, 100 μg. of cellular phosphorus and a 4 hour incubation. The values obtained with uniformly labelled glucose and normal animals have been set at 100, and the other values expressed proportionately (*i.e.* as a percentage).

5. Oxidation of C^{14}-Labelled Glucose by Leucocytes during Phagocytosis.— Leucocytes have been shown to have a considerably increased oxygen uptake during phagocytosis (1, 12), and it was of interest to determine whether there was any concomitant change in the *pattern* of glucose oxidation.

Thus PEC were permitted to engulf heat-killed (100°C.) tubercle bacilli, which had a negligible oxygen consumption, in the presence of specifically labelled glucose. Control experiments were carried out using PEC alone and tubercle bacilli (R1Rv) alone. The results are presented in Table IV. It may be observed that the leucocytes had a greatly increased rate of oxygen uptake during phagocytosis as mentioned above (1). The experiment was terminated after an hour.

From the table it is clear that the oxidation of glucose was enhanced during phagocytosis, as shown by the greatly increased elimination of C_1 of glucose [line 3 in the table]. Elimination of C_6 of glucose was unaltered (*cf.* lines 3 and 4, Table IV). The ratio C_1/C_6 in this case is about 70.

The effect of phagocytosis on the ratio of expiration of glucose C_1 to C_6 becomes even more strikingly apparent when the values obtained for the combined system of phagocytes and dead bacteria (line 3, Table IV) are cor-

rected for those obtained for bacteria alone (line 2). That this is a legitimate correction is indicated by the fact that the values for C_6 of the sum of the separate systems, or for the combined system, are the same (lines 3 and 4). When this correction is made, the ratio C_1/C_6 becomes 131 during phagocytosis. It is thus believed that the direct oxidative pathway provides the main part of the oxidative energy required for phagocytosis.

TABLE IV

Oxidation of Glucose-1-C^{14} and Glucose-6-C^{14} by PEC alone, and during Phagocytosis of Dead Tubercle Bacilli

Cells	O_2 uptake*	Activity in CO_2, c.p.m.		Ratio‡
	μl.	C_1‡	C_6‡	C_1/C_6
(1) PEC	12.7	432	14.5	29.7
	12.7	336	17.2	19.5
(2) R1Rv	1	134	10.3	13.0
	2	123	16.5	7.5
(3) PEC and R1RV together	27.1	1609	23.8	67.5
	27.6	2085	29.2	71.4
(4) Sum of means of values for PEC and R1Rv separately (*i.e.* lines (1) + (2))	14.2	512	29.3	17.5
(5) Mean value for PEC and R1Rv together, corrected for R1Rv alone (*i.e.* lines (3) − (2))	25.9	1718	13.1	131.0

* Oxygen uptake in experiments with PEC expressed as $\mu l.O_2/100$ μg. cellular P and hour.

‡ C_1 and C_6 refer to CO_2 derived from flasks containing glucose-1-C^{14} and glucose-6-C^{14} respectively. Results have been normalized to 1×10^5 c.p.m. used.

It should be noted that 2 per cent serum was present in the medium in these experiments in order to promote phagocytosis. This probably accounts for the fact that the C_1/C_6 ratio is higher than the ratio obtained in the experiments quoted in Table III, in which no serum was present. Lactate present in the serum would, for example, dilute three-carbon units derived from glucose by glycolysis, and thus dilute the radioactivity that is derived from C_6, without affecting the appearance of that from C_1 by the direct oxidative pathway. The very significant appearance of activity from glucose-1-C^{14} brought about by the heat-killed tubercle bacilli alone is as yet unexplained and is under investigation.

DISCUSSION

It is clear from the results reported, that infection of guinea pigs with tubercle bacilli induces in exudate leucocytes a higher respiratory activity.

These observations are compatible with earlier findings that monocytes from exudates of rabbits infected with tubercle bacilli showed greater phagocytic and mitotic activity than those from normal rabbits (13). In addition, it was noted by Lurie (13) that the infected rabbits yielded more MN in their peritoneal cavity upon injection of an irritating agent than did the control animals. It is worth noting that in Lurie's experiments the increase in activity due to infection was significantly greater if the infection was progressive; it was smaller and statistically not significant in the case of infection with an attenuated strain. The same is true for the respiration measurements obtained in the experiments reported in this paper (see Fig. 1).

The observation that there is no change in the pattern of $C^{14}O_2$ production from C_1 or C_6 of glucose, although respiratory activity of the leucocytes is increased, is of interest. The values for the ratio of $C^{14}O_2$ from glucose-1-C^{14}, to that from glucose-6-C^{14} (C_1/C_6) which were obtained ranged from 7 to 13, and are considerably higher than those reported for liver, diaphragm or kidney; *i.e.*, 2.9, 1, and 1.1 respectively (9–11). They are of the same order as figures reported for the corneal epithelium; *i.e.*, 6.7 (14). The figures obtained with the PEC suggest that the hexosemonophosphate shunt (the direct oxidative pathway) is an important pathway of glucose metabolism in these cells, and indeed that the major proportion of the respiratory CO_2 from glucose oxidation is derived by this route, at least under the conditions used in these experiments. PEC do, however, have a very notable aerobic glycolysis (1).

The fact that the ratio of expiration of C_1 to C_6 of glucose was very considerably raised when the leucocyte was stimulated (*i.e.* during phagocytosis), confirms the opinion that the shunt pathway is a major source of metabolic energy. However, it would be of importance to establish whether or not any components of the ingested tubercle bacilli affect the respiration of the leucocyte specifically. Glucose metabolism of leucocytes and the energetics of phagocytosis require further detailed study.

Metabolic differences between normal tissue and the tissue of infected animals have been observed before. The first observations were limited to measurements of over-all metabolism in normal and tuberculous human beings (15). Subsequently, individual enzyme systems have been examined and found to differ in the normal and infected host. For example, enzymes such as phosphatases were found to be increased in the tuberculous granuloma (16, 17) and the tissues themselves showed higher activity. Recently the succinic dehydrogenase activity of tissues from normal, infected and sensitized guinea pigs was measured. The electron transfer system of the tissues exhibited lower activity, the more severe the infection of the animal from which the tissue was taken. Furthermore, organs usually not involved in the tuberculous

process in guinea pigs, such as the kidney, showed this difference to a high degree (18).

In the case of another infection, it has recently been reported that the capsular polysaccharide from pneumococci type I activates the adenosinetriphosphatase activity of guinea pig leucocytes more than 100 per cent above the control in concentrations from 10^{-3} to 10^{-6} per cent (19). It is possible that the higher respiration in PEC from guinea pigs infected with tubercle bacilli is the result of a stimulation of catabolic processes by some component of the tubercle bacilli present in these cells, or that the cells obtained from infected animals contain a greater proportion of young cells. Hormonal influences due to the response of the host to infection can also not be ignored.

The question arises whether these metabolic changes are consequences of the infection with tubercle bacilli, which have no effect on the further course of the infection or whether such metabolic changes do contribute to the state of relative immunity of the host which is acquired after a primary infection. The facts that acquired immunity increases with the degree of residual virulence of the strain used for immunization (20) and that the observed metabolic changes are more pronounced in virulent infections indicate that a causal relationship between the two may exist. This is further substantiated by the fact that the products of metabolic reaction of the host tissue lead to the accumulation of substances which are inhibitory to the tubercle bacillus (21).

With respect to the findings in tissue slices, it may be noted that the increase in respiratory activity of liver slices of infected animals was significant, whereas that of kidney was not. The question could be raised as to whether this result might be due in infected animals to the accumulation in the former tissue of cells of the reticulo-endothelial system; *i.e.*, granulomatous lesions, which were observed (p. 270) in the liver. The Q_{O_2} (μl. O_2/mg. dry weight and hour) of normal guinea pig liver slices was 3.29, that of slices from infected livers was 5.42. Mononuclear leucocytes from guinea pigs have a Q_{O_2} (μl. O_2/mg. dry weight and hour) of 7.4 (1). Thus, invasion of the liver by such cells might be the cause of increased respiration in liver slices from infected animals.

In contradistinction to the behavior of isolated leucocytes and slices of liver, the whole animal showed no observable change in CO_2 production, and glucose oxidation, nor were there any real differences in the pattern of $C^{14}O_2$ production from glucose-1-C^{14}, glucose-6-C^{14}, or glucose-U-C^{14}. Any changes due to the observed effects in liver and PEC would probably be masked by the fact that these tissues contribute only a fraction of the total oxygen consumption and glucose oxidation of the body. It is thus evident that changes in metabolic function of the infected host are best sought in isolated cells or individual tissue preparations.

SUMMARY

In continuing studies concerning the interactions between phagocytes and tubercle bacilli the effect of tuberculous infection on respiration and glucose utilization was investigated in guinea pigs.

Peritoneal exudates rich in polymorphonuclear leucocytes, derived from guinea pigs infected with tubercle bacilli, had a significantly higher rate of respiration than the same cells from normal animals. The difference between cells from normal and infected animals was greater when the animals were infected with a virulent strain (Vallée) than when infected with an attenuated one (R1Rv or BCG). By the use of glucose labelled with C^{14} at position 1 or 6, or uniformly labelled glucose, it was established that this difference in oxygen uptake between normal and infected cells was probably not caused by a difference in the pathway of glucose utilization.

Similarly, the respiration of liver and kidney slices from normal and infected guinea pigs was compared and it was found that liver slices showed differences similar to those shown by leucocytes, but that the kidney slices did not. The possibility has not been ruled out that the difference in rate of respiration of liver slices due to infection might be caused by tuberculous lesions in the livers of infected animals. The mononuclear cells which invade the liver have a higher rate of oxygen uptake than liver cells.

The rate of glucose utilization and the total amount of CO_2 produced was also determined in intact guinea pigs. Both functions were found not to differ significantly in normal and infected animals. The rate of production of CO_2 from C_1 and C_6 of glucose was the same in both groups of animals.

The ratio of the rate of production of $C^{14}O_2$ from C_1 and C_6 of glucose by the whole animal was found to be about 1.35. It was found to be much higher with polymorphonuclear leucocytes ($C_1/C_6 = 8$ in the absence of serum). During the process of phagocytosis this ratio increased from about 25 to about 130 (in the presence of 2 per cent serum) indicating an increase in the direct oxidative pathway of glucose utilization during stimulated cellular activity.

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BIBLIOGRAPHY

1. Stähelin, H., Suter, E., and Karnovsky, M. L., *J. Exp. Med.*, 1956, **104,** 121.
2. Stähelin, H., Karnovsky, M. L., and Suter, E., *J. Exp. Med.*, 1956, **104,** 137.
3. Dubos, R. J., and Middlebrook, G., *Am. Rev. Tuberc.*, 1947, **56,** 334.
4. Van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 1924, **61,** 523.
5. Robinson, C. V., *Science*, 1950, **112,** 198.
6. Garrard, I. D., and Sherman, H. C., *J. Am. Chem. Soc.*, 1918, **40,** 955.
7. Karnovsky, M. L., Foster, J. M., Gidez, L. I., Hagerman, D. D., Robinson, C. V., Solomon, A. K., and Villee, C. A., *Anal. Chem.*, 1955, **27,** 852.

8. Lee, M. O., *Am. J. Physiol.*, 1929, **89,** 24.
9. Agranoff, B. W., Brady, R. O., and Colodzin, M., *J. Biol. Chem.*, 1954, **211,** 773.
10. Ashmore, J., Kinoshita, J. H., Nesbett, F. B., and Hastings, A. B., *J. Biol. Chem.*, 1956, **220,** 619.
11. Bloom, B., and Stetten, DeW., *J. Am. Chem. Soc.*, 1953, **75,** 5446.
12. Baldridge, C. W., and Gerard, R. W., *Am. J. Physiol.*, 1933, **103,** 235.
13. Lurie, M. B., *J. Exp. Med.*, 1939, **69,** 579.
14. Kinoshita, J. H., Masurat, T., and Helfant, M., *Science*, 1955, **122,** 72.
15. Wells, H. G., DeWitt, L. M., and Long, E. R., The Chemistry of Tuberculosis, Baltimore, Williams & Wilkins Company, 1923, 139.
16. Grogg, E., and Pearse, A. G. E., *Brit. J. Expt. Pathol.*, 1952, **33,** 567.
17. Vogel, F. St., *J. Exp. Med.*, 1951, **93,** 305.
18. Martin, S. P., Chaudhuri, S. N., Cooper, C. D., and Green, R., *in* Ciba Foundation Symposium on Experimental Tuberculosis (G. E. W. Wolstenholme and M. P. Cameron, editors), Boston, Little, Brown and Co., 1955, 102.
19. Murayama, M., Doctorate Dissertation, Ann Arbor, University of Michigan, 1953.
20. Trudeau, E. L., Transactions, 1st Annual Meeting National Association Study and Prevention of Tuberculosis, 1905, 157.
21. Dubos, R. J., Biochemical Determinants of Microbial Diseases, Cambridge, Harvard University Press, 1954.
22. Horecker, B. L., and Mehler, A. H., *Ann. Rev. Biochem.*, 1955, **24,** 207.