Effects of *Salmonella* Vaccination on Metabolism and Resistance to Infection of Rabbit Peritoneal Cells*

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Earlier work by one of us (Allison, Zappasodi, and Lurie, 1962b) demonstrated that, after BCG vaccination in the rabbit, there was a period of depression in the metabolism of mononuclear cells from the peritoneal cavity. This period of depression in metabolism was associated with a depression in resistance to tuberculosis (Allison et al., 1962a). Later, the metabolism of the mononuclear cells of the host rose to a level considerably above normal, and during this period the host displayed an increased resistance to tuberculosis. Since other studies (Howard et al., 1959; Shaedler and DuBos, 1957) have shown that BCG vaccination also raises the resistance against a variety of heterologous infections, it was reasonable to assume that this alteration of resistance by BCG might be non-specific. Vaccination by other organisms might possibly produce the same effects on metabolism of mononuclear cells as well as host resistance. This study was designed to show the effect of another vaccine (*Salmonella*) on the depression of cellular metabolism and its corresponding depression of the host's resistance to an acute infectious process.

Materials and Methods

The rabbits used in these experiments were New Zealand white, purchased from local breeders, housed under standard conditions of temperature and environment, and fed on Purina rabbit chow. Water was available ad lib. The rabbits were divided into six groups of four to six animals. Group 1 served as controls for metabolic studies and received no vaccine. Group 2 received subcutaneously 1 ml of killed *Salmonella* vaccine containing 1,000 million typhoid, 250 million paratyphoid A, and 250 million paratyphoid B. Four days post-vaccination, this group was killed, and the peritoneal exudate was used for metabolic studies. Group 3 received two subcutaneous injections of the same standard *Salmonella* vaccine 30 days apart. The animals were killed four days after the second injection; the peritoneal exudate cells were used for metabolic studies. Group 4 received three injections of the standard *Salmonella* vaccine, 30 days apart. The animals were killed four days after the last injection; the peritoneal exudate cells were used for metabolic studies. Group 5 consisted of normal non-vaccinated animals injected intravenously with a suspension of *Candida albicans*. Group 6 was vaccinated with three injections of standard *Salmonella* vaccine spaced 30 days apart, and four days after the last injection these animals were infected with *C. albicans*. The animals infected with *C. albicans* were permitted to live for a maximum period of 10 days. At the 10-day period, the surviving animals were killed. The exudates were obtained using mineral oil as previously described (Allison et al., 1961) and all animals were killed by air embolism. The first four groups were used for

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metabolic measurements; the last two for resistance studies.

The enzyme studies were done with the Thunberg technique as described before (Allison et al., 1961). The following substrates were used: lactic acid, sodium succinate, sodium glycerophosphate, DL-β-hydroxybutyric acid, malic acid, glycerol, and α-keto-glutaric acid. Simultaneous measurements of endogenous respiration were also performed. Additional metabolic studies were performed, in some cases, using Smithies' vertical starch-gel method (Smithies, 1959) to separate the enzyme lactic dehydrogenase into its various isozyme components. The enzymatic activity was measured by a modified Nachlas substrate and quantitated with a microscope photometer scanner previously described (Allison, Gerszten and Sanchez, 1963).

The culture used to test the resistance of the animals was a strain of *C. albicans* recently isolated from a human case of candidiasis. The animals received a dose of 60 million organisms from a four-day growth of the *Candida* culture. When the animals died, a complete autopsy was done, and sections were taken for microscopic examination. Gomori-methanimine silver nitrate stain was used to visualize the *Candida* organisms in the tissue; hematoxylin and eosin stains were also done.

Results

The peritoneal exudates from the various groups of rabbits showed essentially the same numbers of total cells recovered and the same type of differential pattern. Approximately 600 $\times$ 10^6 cells were recovered for each rabbit; 97% of these cells were mononuclears.

Table I presents the values for the metabolic studies done on the peritoneal exudate cells. These values were expressed as the slope of the line for the reduction of methylene blue per 10 million exudate cells. It is evident from this table that a single injection of *Salmonella* vaccine causes a drastic reduction in the general metabolic activity, of a number of dehydrogenases, four days post-vaccination. The administration of a second injection of *Salmonella* vaccine approximately 30 days later caused a further drop in activity with most of the substrates studied. This period seemed to be the lowest point in metabolic activity, for a

TABLE 1

Metabolic Studies on Peritoneal Exudate Cells. Values Per 10 Million Cells.

		Lactate	Succinate	Glycero-phosphate	β-oh-butyrate	Malate	Glycerol	α-keto Glutarate	Endogenous
	Obser.	5	5	5	4	5	5	5	5
Group 1 Control	Activity	.089 ±.009	.085 ±.016	.105 ±.008	.058 ±.007	.026 ±.010	.043 ±.009	.025 ±.003	.021 ±.006
	Obser.	4	4	4	4	4	4	4	4
Group 2 Experimental	Activity	.048 ±.014	.040 ±.012	.065 ±.008	.044 ±.019	.009 ±.0005	.021 ±.019	.005 ±.004	.038 ±.004
	Obser.	4	4	4	4	4	4	4	4
Group 3 Experimental	Activity	.036 ±.016	.031 ±.013	.031 ±.011	.021 ±.008	.012 ±.010	.011 ±.006	.010 ±.007	.014 ±.004
	Obser.	5	5	5	5	5	5	5	5
Group 4 Experimental	Activity	.035 ±.007	.034 ±.007	.049 ±.008	.021 ±.005	.014 ±.007	.017 ±.003	.019 ±.006	.004 ±.002

Group 1—Non-vaccinated.
Group 2—1 dose *Salmonella* vaccine. 4 days post-vaccination.
Group 3—2 doses *Salmonella* vaccine. 4 days post-vaccination.
Group 4—3 doses *Salmonella* vaccine. 4 days post-vaccination.

third injection given 30 days after the second did not cause any further decline in the metabolic activity of these cells.

In an attempt to determine whether this reduction in enzyme activity after vaccination with *Salmonella* was due to loss of enzyme or simply loss of some co-factor, extracts of the peritoneal exudate cells in vertical starch-gel were electrophoresed. They were then exposed to a lactic acid substrate using phenazine methosulfate and diphosphopyridine nucleotide as the electron accepter. An average value of 492 units was found for the control and 423 for the experimental, a difference which was not significant. Thus the reduction in activity must be due to a reduction or loss of co-factors rather than a loss of enzyme itself.

Since this experiment served to strengthen further the correlation of resistance to infectious disease with metabolic activity of mononuclear cells, evaluation was needed of the resistance of these animals whose peritoneal exudate cells were at a very low metabolic level.

The experimental rabbits vaccinated with *Salmonella* all died earlier than 60 hours post-infection with *C. albicans*. The non-vaccinated control animals lived for periods over 60 hours after infection, some surviving for as long as 10 days.

The vaccinated rabbits infected with *C. albicans* died of an acute generalized pneumonic process characterized by massive edema, severe congestion, and a moderate amount of macrophage infiltration. The control rabbits, on the other hand, exhibited a moderate interstitial pneumonia, but the principal lesions were located in the kidneys. These consisted of multiple, pinpoint abscesses. These lesions were not visible grossly in the experimental animals, although a small number were identified microscopically. Typical lesions found in the lungs and kidneys are illustrated in figures 1 and 2.

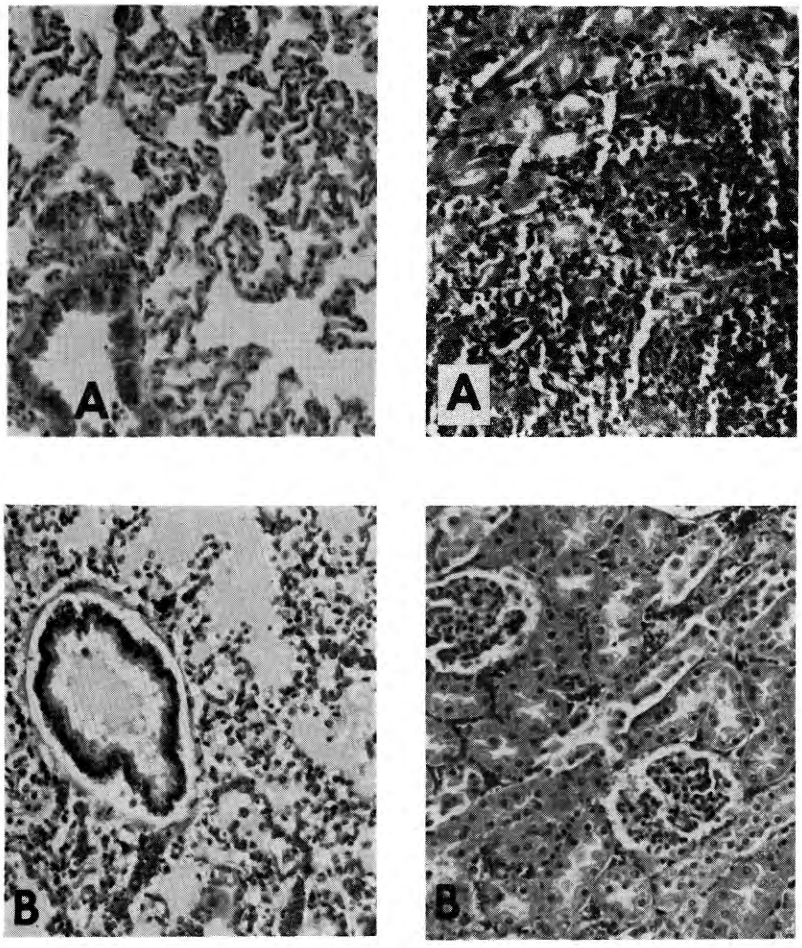

Fig. 1—Pulmonary findings (140X) in the rabbit. (*A*) Control, with minimal interstitial pneumonitis. (*B*) Experimental, with severe pulmonary edema, congestion and early bronchopneumonia.

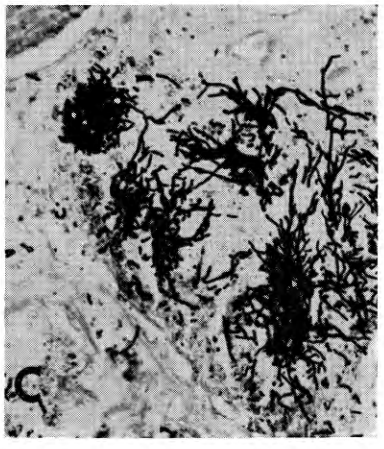

Fig. 2—Candidiasis of the kidney (140X) in the rabbit. (*A*) Control, with extensive abscesses. (*B*) Experimental, with minimal infection. (*C*) The growth of the organisms in the control kidney is brought out by the G.M.S. stain.

The main pathological findings in the control animals were in the kidneys; in the vaccinated animals the major changes were in the lungs.

Discussion

Previous studies (Allison et al., 1962a and b) have demonstrated that in tuberculosis there is a close correlation between resistance of the animal and the level of metabolic activity of peritoneal mononuclear exudate cells. This study was concerned with the reduction in metabolism associated with a reduction in resistance to an acute disease such as candidiasis. The observations on the metabolism of mononuclear peritoneal exudate cells following *Salmonella* vaccination would indicate that a reduction in metabolism occurs at about the same time as that following vaccination with BCG. This reduction in metabolism is also associated with a reduction in resistance of the host to a heterologous acute infection caused by *C. albicans*.

The candidiasis in the experimental animals was primarily a pulmonary disease, whereas in the controls it was a severe kidney disease. Histopathological studies tend to support the findings of Evans and Winner (1954) with regard to the kidney lesions, but not with regard to the pulmonary lesions. The severe pulmonary edema that was a constant feature in the experimental animals at the time of death was an integral part of the disease process and could not be attributed to outside influences such as chloroform (as suggested by Evans and Winner). The presence of extensive pulmonary edema can be associated with the increased susceptibility of these animals, and this pulmonary disease was the primary cause of death. It would appear that the kidney disease as the main focus of the infection would depend on the host's living for longer periods of time. Studies in mice by Hurley and Winner (1963) would also tend to support this hypothesis. The lengthened life span could be attributed to a greater resistance of the host, as in our case, or a reduction in the number of organisms injected, as in the studies of Hurley and Winner.

In previous studies, one of us (Allison et al., 1962b) noted that this reduction in metabolism was not due to a loss of enzyme, since reactivation of the enzymatic activity was possible by the addition of heated mononuclear cells from normal animals. In this present study we demonstrated that loss of enzyme was not the factor responsible for the reduction of the metabolic activity. If phenazine methosulfate was used as the hydrogen accepter, no difference in activity of the enzymes was noted between control and experimental animals. This work, and previous work using BCG as the vaccine, would tend to support a clinical impression among some physicians that patients are often more susceptible to colds, influenza, and other infectious diseases shortly following vaccination or immunization procedures. The mechanism of this reduction in resistance may be related to a depression in certain enzymes of the host due to the action of the vaccine, and a study of human leukocytes following immunization may be of value.

Summary

Salmonella vaccine caused a depression of metabolism of peritoneal exudate cells from rabbits. This effect was associated with a depression in resistance of rabbits to infection with *C. albicans*. This depression in metabolism is similar to the one previously noted following BCG vaccine and associated with a depression in resistance to tuberculosis.

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