In attempting to determine the relation of various chemical components of tubercle bacilli to the disease itself, we have used both prophylactically and therapeutically the lipoids and residues obtained by Anderson (1). In the present communication the prophylactic properties of defatted bovine tubercle bacilli will be discussed.

Attempts to immunize actively or passively against tuberculosis were begun soon after the discovery of the etiological agent by Koch. It is now generally accepted that passive immunization is unsuccessful and that active immunization is relative rather than absolute. Moreover, most of the agents used for active immunization thus far have possessed certain toxic properties. For the successful prophylaxis against tuberculosis, therefore, search must be made for a relatively non-toxic substance of high antigenic potency. The only agents as yet discovered, which in any measure fulfill these requirements, are tubercle bacilli.

Koch himself was probably the first to employ tubercle bacilli in an attempt to immunize against tuberculosis. More recently, Webb and Williams (2) have demonstrated increased resistance to tuberculosis in monkeys vaccinated with small doses of living virulent organisms. Selter likewise used living bacilli (3). These procedures, together with the introduction of attenuated bovine bacilli, namely, BCG, by Calmette and Guerin (4), have precipitated a vigorous controversy over the efficacy and safety of such prophylactic measures. Petroff demonstrated that killed tubercle bacilli induced hypersensitiveness to tuberculin (5) which persisted as long as 470 days (6). He proposed the use of heat-killed organisms as a measure accompanied by less danger than the use of viable forms and gave a review of the literature on tuberculosis vaccination (7). Also tubercle bacilli have been submitted to many chemical procedures in the search for a suitable immunizing agent. Prophylaxis with formalin-treated organisms was
discussed by Löwenstein (8) in 1913. Dreyer (9) treated the organisms first with formalin, then extracted with acetone while heating, and repeated these procedures until the bacteria were no longer acid-fast. The diaplyte vaccine thus prepared was first thought to be quite efficacious and was employed clinically, as well as experimentally. Bronfenbrenner and Straub (10), and Douglas, Eddington, and Simson (11), however, found no beneficial effect from vaccination by Dreyer's method.

The vaccine used in the present experiments was prepared in the following manner.

Bovine tubercle bacilli were grown on Long's synthetic media for 3 weeks. To the bacilli, after they were harvested, equal parts of ether and 95 per cent alcohol were added. The mixture of ether, alcohol, and bacilli was saturated with CO₂ and allowed to stand with casual shaking for 4 months and 22 days. The alcohol-ether mixture was then decanted, the organisms were dried in the air, and ground for 48 hours in a ball mill. This fine powder was prepared by the Mulford Laboratories, Sharp and Dohme, Glenolden, Pennsylvania and was obtained through the courtesy of Dr. John Reichel. It contained a moderate number of intact bacilli, some of which retained their acid fastness. No viable tubercle bacilli could be demonstrated by cultures or inoculation of animals. This powder was ground thoroughly in a mortar with sterile 0.9 per cent saline, so that 1 cc. of the final suspension represented 0.2 mg. of the partial defatted organisms.

EXPERIMENTAL

Thirty rabbits were used in the experiment. Total and differential counts of the blood cells were made before any experimental procedure was begun. The differential counts were made by the supravital method. The weight of each animal was determined. After an adequate preliminary study of the blood had been made, ten of the animals were vaccinated with the partially defatted bacilli described above. Injections were done every 3rd day until each animal had seven injections. The volume of the suspension was in each instance 1 cc. At the time of the first injection each animal received 0.1 mg. of the partially defatted bacilli. The amount of each subsequent injection was 0.2 mg., so that each animal received a total of 1.3 mg. Half of the animals were vaccinated subcutaneously; the others intravenously. Blood studies were continued during and after the period of vaccination. 3 months and 8 days following the last injection of vaccine, the test and their control animals were inoculated intravenously with 0.1 mg. (moist weight) of a 16 day old subculture of bovine tubercle bacilli, Strain B-1. The animals were weighed and their blood cells were counted at irregular intervals from the time of inoculation until death. An autopsy was done on each animal and surveys were made of the macroscopic and microscopic pathology with special reference to the extent, distribution, and character of the lesions.
RESULTS

One vaccinated animal died 2 days following the last subcutaneous injection of the vaccine. From the autopsy, the fixed tissues, and from bacterial stains, it was found that the animal had a non-tuberculous pneumonia and empyema. In addition, one intravenously vaccinated animal was killed 15 days after the seventh injection for a study of the reaction to the defatted tubercle bacilli. The sections of lung from this animal showed numerous small masses of epithelioid cells surrounded by lymphocytes and a few giant cells, mostly of the foreign body type. The lesions were regressing, as many of the epithelioid cells showed evidence of degeneration. Tiny foci of caseation were present in some of the tubercles. A small number of rosette giant cells were seen also in the liver and spleen. The bone marrows were depleted and showed many abnormally young erythroid and myeloid cells. The latter observation was of interest in view of the changes in the blood cells due to the vaccinations.

Each of the animals vaccinated subcutaneously (in the groin) exhibited typical cold abscesses at the site of these vaccinations. In some instances these abscesses ruptured; in all instances the abscess or ulcer was healed at the time of inoculation with living tubercle bacilli.

Analyses of the blood counts of the vaccinated rabbits in the interval between vaccination and inoculation showed a most interesting sequence of events. All of the vaccinated animals developed an anemia and a leucopenia similar to that described by Sabin, Doan, and Cunningham (12), and by Sabin and Doan (13). The fall in total red and total white cells began during the vaccination period and continued 10 days after the last injection, after which there was a gradual return to values slightly higher than normal. The average total fall in red blood cells was about 1.4 millions (Chart 1); of the white cells 2,800 cells (Chart 2). Upon recovery the average white blood cell counts reached and were maintained at a level 2,500 cells higher than that existing before vaccination. From Charts 3 and 4 it will be seen that this overcompensation was due in part to higher values for granulocytes, but principally to a well marked lymphocytosis. Chart 4 also shows that the values for lymphocytes were maintained through-
out the course of the disease at a level very significantly above that of the controls. The other types of white blood cells showed no significant difference from the controls during the postinoculation period. The partially defatted tubercle bacillus vaccine therefore contained the factors present in living bacilli which cause the anemia and leucopenia. In addition, there was the capacity to cause lymphocytosis that has been shown by Thomas to occur after the intravenous inoculation of rabbits with living tubercle bacilli (14, Fig. 6). In the present instance this lymphocytosis was sustained for a prolonged period.

The animals vaccinated with the partially defatted tubercle bacilli showed a very definite increase in resistance after inoculation with virulent living organisms. The average survival of the control animals was 167 days, while that for the vaccinated animals was 249 days—an increase of about 48 per cent. Moreover, the average survival time of the animals vaccinated intravenously was 319 days—an increase of 90 per cent above that for the controls. This was particularly significant in that the only intravenously inoculated animal which died under 100 days suffered a complicating non-tuberculous encephalitis (14). On Chart 5 is recorded the incidence of deaths in the vaccinated and control groups, by intervals of 15 days. This chart also shows the average survival of the two groups of animals.

The study of the lesions in the vaccinated and control animals showed that the former had somewhat fewer and less extensive lesions than the controls. Careful analysis with particular attention to the phase of the disease in which the animals died showed that this difference was not due to the diphasic nature of the disease (14). The greatest differences in extent and incidence of lesions were noted in the lymph nodes and bone marrow. In the vaccinated rabbits, only 10 of 51 lymph nodes were tuberculous (19.6 per cent). In the controls, 51 of 118 lymph nodes were tuberculous (42 per cent). In the vaccinated animals, 6 of 23 bone marrows (26 per cent) were tuberculous, whereas in the controls, 25 of 54 marrows exhibited the lesions (46 per cent). Furthermore, from Table I it will be seen that the vaccinated animals dying in the second 100 days of the disease showed no bone marrow or lymphadenoid tuberculosis, while lesions were common in the controls at this stage. Since bone marrow and
CHART 1. Graph of the circulating red blood cells of the vaccinated and control animals; the curves for both groups of vaccinated animals cover the periods before, during, and after vaccination as well as after inoculation with living tubercle bacilli, while that for the controls begins immediately before inoculation. The values on any day are expressed as averages for each group.
CHART 2. Graph of the circulating white blood cells of the vaccinated and control animals; the curves for both groups of vaccinated animals cover the periods before, during, and after vaccination as well as after inoculation with living tubercle bacilli, while that of the controls begins immediately before inoculation. The values are expressed as averages for each group. The sharp rise and fall in total leucocytes of the subcutaneously vaccinated animals were caused by an unexplained extreme leucocytosis in one of the group.
CHART 3. Graph of the circulating neutrophils of the vaccinated and control animals; the curves for both groups of vaccinated animals cover the periods before, during, and after vaccination as well as after inoculation with living tubercle bacilli, while that of the controls begins immediately before inoculation. The values shown are expressed as averages for each group.
Chart 4. Graph of the circulating lymphocytes of the vaccinated and control animals; the curves for both groups of vaccinated animals cover the periods before, during, and after vaccination as well as after inoculation with living tubercle bacilli, while that for the controls begins immediately before inoculation. The values shown are expressed as averages for each group for any one day. Note the sustained high level of lymphocytes in the vaccinated animals as compared with their own normal values and those of the controls before and after inoculation.
lymph node lesions which occur after the 3rd month of the disease may be considered as metastatic (13), often arising from vascular lesions in remote parts of the body (15), it would seem that the power to localize the lesions was enhanced in these vaccinated animals. However, this power to localize the lesions was by no means absolute, as animals which died later in the disease showed numerous metastatic foci of tuberculosis.

![Chart 5](image)

**Chart 5.** Incidence of deaths in the vaccinated and control groups in each 15 day period of the disease and the average survival time of the two groups in days.

It may be well to mention at this point that the vascular lesions referred to above, and previously described as occurring in the acute phase of the disease (15), were by no means limited to that phase. In the present experiments venous thrombi were seen in twelve animals—in one instance as late as 498 days after inoculation. These lesions were seen to occur either by direct extension of tubercles through the wall of a vein or by obstruction of lymphatic vessels by epithelioid cells and subsequent extension through the vein wall. Such vascular lesions were invariably present in those animals living longer than 3
months which showed tubercles in the bone marrows and peripheral lymph nodes.

Since Petroff (6) had shown that heat-killed tubercle bacilli render guinea pigs sensitive to tuberculin, it was decided to determine whether the partially defatted organisms possess this property. Accordingly, twelve non-tuberculin-reacting guinea pigs were divided into six pairs of two. One pair received a single intraperitoneal injection of 2.5 mg. of the defatted tubercle bacilli (human Strain H-37), one pair received two injections, and the other pair received three injections, each spaced by intervals of 5 days. The remaining six guinea pigs each received similar injections of heat-killed human tubercle bacilli. 28 days following the first injections, all the guinea pigs were tested with 0.1 mg. tuberculoprotein MA-100 intracutaneously. All tests were definitely positive. These were repeated 33 days later and were again positive, some more strongly than at the time of the first test. It is clear then that the defatted, as well as the heat-killed, tubercle bacilli can induce cutaneous hypersensitivity to tuberculoprotein. Moreover, a single injection of 2.5 mg. of either is sufficient to sensitize.

The prophylactic value of the defatted tubercle bacillus vaccine is being studied more extensively at the present time.

### Table 1

<table>
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<td></td>
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<td>Controls</td>
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SUMMARY

1. A vaccine prepared from partially defatted bovine tubercle bacilli influenced favorably the survival time after inoculation with living virulent bovine tubercle bacilli.

2. Accompanying this increased resistance was a sustained lymphocytosis in the vaccinated animals.

3. The vaccine induced a transitory anemia and leucopenia.

4. A similar preparation of partially defatted human tubercle bacilli possessed the power to sensitize guinea pigs to tuberculo-protein.

BIBLIOGRAPHY