THE MINERAL CONSTITUENTS OF THE TUBERCLE BACILLI.

By E. A. de Schweinitz and Marion Dorset.

Received April 14, 1898.

In August, 1895,[1] the writers published the results of some analyses showing the composition of the tubercle bacilli when grown upon different media. Depending upon the character of the media used, the amount of ash varied from two to four per cent. It seemed desirable to make a careful analysis of the ash in order to see which of the mineral constituents of the animal body would be most largely utilized by the germ and consequently necessary for its satisfactory development. The bacilli used for this work had been grown upon neutral beef broth containing one per cent. of peptone, one-half per cent. salt, and seven per cent. glycerol. The cultures, after heating in order to kill the germs, were filtered and washed well with boiling water. The washed bacilli were then dried over sulphuric acid, finely powdered, and thoroughly extracted with pure ether and ninety-eight per cent. alcohol. After the last extraction the bacilli were again dried and ignited at a low red heat until practically all the carbon had been burned. The ash, which was almost pure white in color, was dried to a constant weight at 100° C. The total ash available for analysis was 1.453 grams. Examination showed that sulphates, chlorides, and carbonates were not present in the ash. The method used for the determination of the constituents of the ash were those prescribed for the analyses of the ash of plants. The results calculated upon the dry ash were as follows:

	Per cent.
Na_2O	13.62
K_2O	6.35
CaO	12.64
MgO	11.55
C and Si	0.57
P_2O_5	55.23

The high percentage of phosphorus pentoxide and the absence of other acid radicals in this ash are very noticeable. While it is probable that some of the chlorides and sulphates

[1] This Journal, 17, 605.

may have been washed out of the germ in the process of preparing it for analysis, no chlorides were present in the germs after washing; the fact that the amount of phosphoric acid obtained in the ash is slightly lower than the total amount of phosphoric acid obtained from the whole germ, would indicate that chlorides and sulphates are practically of no importance in the composition of the germ, while their presence in the culture media in minute quantity appears to be necessary for the satisfactory development of the germ. Chlorides and sulphates if dissolved out would have been present probably as cell contents rather than as part of the germ.

Ash analyses of comparatively few germs have been made, and the only ones which give data that may be reported here are the analyses made by Cramer,[1] who found that the composition of the ash of the cholera germs varied greatly, depending upon the quantity of sodium chloride and sodium phosphate that were used in the preparation of the media. In normal media the results were as follows:

	Per cent.
Cl	17.02
P_2O_5	20.48
SO_4	8.55
K	6.32
Na	32.06
Ca	0.98
Mg	trace

If the amount of sodium chloride in the media was increased, the percentage of chlorine in the ash was more than doubled, while the percentage of SO_4 found was reduced to one per cent., and the percentage of P_2O_5 was largely diminished, being reduced to 9.64 per cent. When sodium phosphate was added to the media the percentage of chlorine was found to be 9.99 per cent., the percentage of P_2O_5 34.30 per cent., SO_4 2.24 per cent., of potassium 4.97 per cent., of sodium 31.83 per cent., of calcium 1.29 per cent., of magnesium 0.12 per cent. These results differ greatly from those found in the examination of the ash of the tubercle bacilli. As noted above, the media used for the growth of these latter germs was a normal material containing one-half per cent. of salt, but without the addition of any

[1] *Archiv. für Hygiene*, 28, No. 1.

phosphates or other salts. Consequently the high percentage of phosphorus pentoxide can be attributed only to the fact that phosphorus as well as the calcium and magnesium are absolutely necessary for the development of the tubercle bacilli, and were derived by it from these elements as normally present in the media.

In arrested cases of tuberculosis in animals, we often find hard, gritty, calcareous nodules. These nodules in healed tuberculosis contain tubercle bacilli. In other cases of healed tuberculosis where calcareous nodules are not present no bacilli as a rule are found. It is easy to trace a very close connection between these nodules in healed tuberculosis, and the composition of the ash of the germ.

The high percentage of fat contained in the body of the tubercle bacilli, which we have noticed in previous papers, in conjunction with this high percentage of calcium and magnesium phosphate in the ash, give grounds for some interesting speculation. Phosphates and cod-liver oil are two materials always strongly recommended in cases of tuberculosis. As the germs of this disease seem to demand a large quantity of food containing phosphorus and also rich in fat, it is but a fair supposition that in giving the drugs above mentioned, we are supplying to the animal body those constituents which are very important for its proper nourishment, the supply of which is constantly being levied upon by the germs of the disease. The question might be asked whether in this method of treatment we are not really feeding the bacilli rather than the individual. But just as an exhausted soil can be made valuable by the addition of constituents which are deficient, so we may assume that the administration of specific materials containing the elements that the germ has utilized, should act in a similar way in increasing the vitality in the animal body. These of course are speculations, based, however, on certain known data. We trust that a still further study which is in progress, including the albuminoid constituents of the tubercle bacilli, may throw some light upon their development and chemical action in the animal body.

BIOCHEMIC LABORATORY,
WASHINGTON, D. C.