

In the third instance two tracks appear below the lead plate. The alternative interpretations are:

(1) a positive particle of small mass and another positive particle emerge from the same point in the lead; or

(2) a 4,000,000 volt electron rebounds from the lead producing the second track; but here a difficulty is met with, since a change in the sign of the charge would have to be assumed to take place in the rebound of the electron; or

(3) the chance occurrence of two independent tracks.

For the interpretation of these effects it seems necessary to call upon a positively charged particle having a mass comparable with that of an electron, or else admit the chance occurrence of independent tracks on the same photograph so placed as to indicate a common point of origin of two particles. The latter possibility on a probability basis is exceedingly unlikely.

The interpretation of these tracks as due to protons, or other heavier nuclei, is ruled out on the basis of range and curvature. Protons or heavier nuclei of the observed curvatures could not have ranges as great as those observed. The specific-ionization is close to that for an electron of the same curvature, hence indicating a positively-charged particle comparable in mass and magnitude of charge with an electron.

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PLASMA PHOSPHATASE IN DAIRY COWS SUFFERING FROM FLUOROSIS

FLUORINE, when included in experimental diets of animals, has been shown to cause a marked disturbance in bone and tooth metabolism. Robison¹ in 1923 showed that an enzyme capable of splitting phosphoric acid esters was instrumental in bone deposition. Kay² in 1930 brought forth evidence to show that the plasma phosphatase increased in such bone diseases as rickets and osteomalacia. The possibility of a change in plasma phosphatase in chronic fluorine poisoning seemed likely, and might offer a means of detecting fluorosis in cases where gross symptoms were not in evidence.

Blood samples were taken from heifers during their first lactation as follows: (1) prior to parturition; (2) near the peak of production; (3) mid-lactation or later; (4) and again near the end of lactation. Six lots of 3 animals each were available. Three of these were lots receiving no known source of fluorine, while the three remaining lots received approximately .02

per cent., .04 per cent. and .087 per cent. of the grain ration as fluorine fed as a mineral supplement in the form of raw rock phosphate. The rations were balanced as to protein. They contained ample energy and were in all respects adequate dairy rations.

The plasma phosphatase was determined by the method of Kay³ except that pH values were determined by means of the quinhydrone electrode rather than by the colorimetric method. In all cases the animals receiving fluorine showed a distinct rise in the plasma phosphatase values over that of the control cows. In nearly all cases the values for our control animals were within the normal range of .1000 to .2000 units per cc for mature animals. Twenty-eight determinations made upon control cows gave a range of from .1168 units to .2440 units per cc with a mean value of .1763 units. The low fluorine lot gave an average phosphatase value of .2366 units per cc. The intermediate group showed a further rise in phosphatase with an average of .2751 units per cc, while the high fluorine lot varied from .2240 units to .5312 units per cc. The mean value for this lot (12 analyses) was .3366 units per cc or practically double that of the control animals. It would seem, therefore, that in cattle suffering from fluorosis the plasma phosphatase rises in proportion to the level of fluorine intake, or nearly so. Other blood constituents, such as serum calcium, inorganic phosphorus, total phosphorus, lecithin phosphorus and chlorine, remained within the normal range. There seemed to be a tendency for the serum calcium of the blood to decrease with a correspondingly slight increase in inorganic phosphorus in the animals most severely affected.

The plasma phosphatase value is apparently an excellent index of the degree of fluorosis in cattle. Not only was there a definite gradation in the plasma phosphatase content between lots, but there was a progressive rise in plasma phosphatase in the high fluorine lot coincident with a progressive severity of the gross symptoms in the cattle. These changes are no doubt explained by the increased grain required to meet the needs of lactation and subsequent higher fluorine ingestion. Cows on the high fluorine (.087 per cent. of the grain ration) gave the following average plasma phosphatase values: at parturition 0.2787 units per cc; near the peak of production, 0.3142 units per cc; mid-lactation, 0.3537 units per cc; and at the close of the first lactation, 0.4227 units per cc. In the absence of other bone diseases the plasma phosphatase in fluorosis forms a sensitive test for the toxic effects of chronic fluorine poisoning. Similar results have also been obtained with swine and rats, although the data with these species are as yet inadequate. The rise in plasma phosphatase indicates

¹ R. Robison, *Biochem. Jour.*, 17: 286, 1923.

² H. D. Kay, *J. Biol. Chem.*, 89: 249, 1930.

³ H. D. Kay, *J. Biol. Chem.*, 89: 235, 1930.

a marked disturbance in bone and tooth metabolism caused by chronic fluorine poisoning. No doubt it represents on the part of the animal body an effort to maintain and deposit normal skeletal structures. There seems to be a marked stimulation to elaborate this enzyme in the case of chronic fluorine poisoning similar to that found in such bone diseases as rickets and osteomalacia.

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MICRO MOTION PICTURES OF *B. SHIGAE* GROWING UNDER CONDITIONS FAVOR- ING FILTERABILITY AND LIFE CYCLE FORMS

Two of the most important problems in present-day bacteriology deal with the reality of bacterial life cycles and the existence of a minute filter-passing form of bacterial life. With a simple and inexpensive micro motion picture apparatus developed primarily for following the action of X- and ultra-violet rays on bacteria, yeasts and molds we have accumulated records of the growth of the dysentery bacillus (*B. shigae*) which bear directly on both questions.

These records, on 6,000 feet of film and covering a period of 600 hours, picture the growth of *B. shigae* in microculture during and after cultivation in ordinary beef infusion media, in media consisting exclusively of either peptones or proteins (gelatine or K media) and in media containing varying quantities of lithium chloride. They show the formation and the subsequent fate of the various abnormally shaped cells which have previously been described as typical stages in a bacterial life cycle. Hadley¹ has filtered LiCl-treated Shiga cultures and Kendall² has stated that the use of his K medium gives filterable stages to the related *B. typhosus*. Many photographs have been made of "filterable" cultures obtained in these ways.

Shiga bacilli when rapidly multiplying in ordinary beef infusion media are rods about $5\mu \times 1\mu$, but by aging these cultures or by changing their food substances it is easy to obtain all the supposed life cycle forms. Real branching occurs early in many special media; in old cultures growth practically always takes place from long filamentous cells. Except for the micrococci to be mentioned later all the other life cycle forms appear merely as stages in the adaptation of the bacillus to new and somewhat unfavorable

media. When in an environment which permits of their growth and multiplication the progeny of these organisms always tend to become more and more normal in shape and size. None of them has shown any special function analogous to the more elaborate reproductive processes of the molds.

The micrococci split from certain bacilli constitute the only type of *B. shigae* growing under the conditions of these experiments which the pictures may not have completely described. These spheres, having a diameter about equal to that of the cells from which they arise, are present in nearly all cultures but are most numerous after several LiCl passages. Many appear in the photographs and some have been followed for long periods of time. No growth has ever been seen and it must be concluded that if they are alive and not mere bits of coagulated protoplasm eliminated by injured organisms, their lag period is long.

In discussing filterability through porcelain filters, a distinction may be drawn between quick and slow outgrowths, or reversions, in the filtrates. By quick reversions are meant those which show massive growth after 48-72 hours; in the slow reversions a period of weeks is required before many bacteria can be found. All efforts to get slow reversions in microculture have thus far failed. Many filtrates have been incubated and examined for periods of two and three weeks but in none of them have we been able to see or photograph anything which could properly be interpreted as alive.

More positive information has been gained concerning the quick reversions. Growth of Shiga in the K medium of Kendall³ results in a large number of small, almost coccoid, cells. Similar dwarfed bacteria are present in LiCl cultures during those passages when they are supposedly filterable. Whenever a quick reversion was observed after filtration through a candle tested by the usual methods, it was always possible to find a few such stunted organisms in the fresh filtrate. After a more or less prolonged lag period they always grew into and multiplied as normal Shiga bacilli. This fact combined with the recent observation⁴ that K medium itself aids the passage of normal bacilli through a Berkefeld filter seems to offer an adequate explanation of the quick reversions.

The details of these experiments will appear in the *Journal of Experimental Medicine*.

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¹ P. Hadley, E. Delves and J. Klimek, *Jour. Infect. Dis.*, 48: 1, 1931.

² A. I. Kendall, *SCIENCE*, 74: 129, 1931; 75: 295, 1932.

³ A. I. Kendall, *Northwestern Univ. Bull.*, 32: 8, 1931.

⁴ P. L. Varney and J. Bronfenbrenner, *Proc. Soc. Exp. Biol. Med.*, 29: 804, 1932.