COMPARATIVE STUDIES ON RESPIRATION.

XV. THE EFFECT OF BILE SALTS AND OF SAPONIN UPON RESPIRATION.

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It was pointed out by Osterhout¹ that substances which increase the electrical conductivity of tissues are antagonized by those which decrease it. An illustration of this is seen in antagonism between NaCl (which increases conductivity) and Na taurocholate² (which decreases it). This case has additional interest as an example of antagonism between anions as well as of antagonism between organic and inorganic substances.

The experiments on electrical conductivity indicated that Na taurocholate acts like CaCl₂ in decreasing conductivity and in antagonizing NaCl. Whether this similarity would be found if other criteria were used remained an open question.

The experiments of the writer³ showed that, when respiration is employed as a criterion, CaCl₂ antagonized NaCl. Similar results were obtained by Gustafson.⁴ In view of this it became especially interesting to study the behavior of tissues under the influence of Na taurocholate.

In the experiments which were made for this purpose the organism studied was *Bacillus subtilis* and the technique was similar to that described in previous papers.⁵ The results are shown in the figures.

Fig. 1 shows the effects of sodium taurocholate (0.0000125, 0.000015, 0.001, 0.0025, and 0.01 M) upon the rate of respiration (expressed as per cent of the normal rate). This organic salt is similar in its

¹ Osterhout, W. J. V., *Science*, 1915, xli, 255.

² Osterhout, W. J. V., *J. Gen. Physiol.*, 1918-19, i, 405.

³ Brooks, M. M., *J. Gen. Physiol.*, 1919-20, ii, 5.

⁴ Gustafson, F. G., *J. Gen. Physiol.*, 1919-20, ii, 17.

⁵ Brooks, M. M., *J. Gen. Physiol.*, 1919-20, ii, 5, 331.

toxicity to lanthanum nitrate in that very dilute solutions produce pronounced effects. There is slight increase in the rate in 0.0000125 M; the respiration is normal in 0.000015 M, while in higher concentrations there is a decrease in the rate. In calculating the molecular

FIG. 1. Curves showing the rate of respiration of *Bacillus subtilis* (expressed as per cent of the normal) in 0.0000125, 0.000015, 0.001, 0.0025, and 0.01 M Na taurocholate. The zero point on the abscissa denotes the beginning of exposure to the salt solution; previous to this the bacteria were in 0.75 per cent solution of dextrose in distilled water. The normal rate (which is taken as 100 per cent) represents a change in pH value from 7.78 to 7.60 in a number of seconds depending upon the amount of bacterial suspension used, usually 30 seconds. Each curve represents a single typical experiment.

concentrations of this salt an approximate estimate only could be made because the purity of the salt is doubtful. In making up the solutions 1 gm. of sodium taurocholate in 100 cc. of distilled water was calculated to make about 0.02 M. During the first 10 minutes the

bacteria are under normal conditions and the curve (broken line) is horizontal. After this (at the point marked 0 on the abscissa) the salt is added. For example, the addition of sufficient Na taurocholate to make the concentration 0.0000125 M produces a rise in the rate, which remains constant during the period of experimentation. These curves are selected from a number of similar typical curves, and each represents one experiment.

FIG. 2. Curve showing the rate of respiration of *Bacillus subtilis* (expressed as per cent of the normal) as affected by Na taurocholate. Normal rate as in Fig. 1. Average of three experiments; probable error of the mean less than 2 per cent of the mean.

Fig. 2 shows the effects of various concentrations of sodium taurocholate upon the rate of respiration, expressed as per cent of the normal rate. The rate indicated is that produced after the bacteria had been in contact with the salt for 1 hour. The figure shows that sodium taurocholate produces an increase in the rate at a concentration of 0.0000125 M and a decrease in concentrations higher than 0.000125 M. It is of interest to note that the concentrations which are most favorable to the respiration of *Bacillus subtilis* are very dilute. The abscissæ represent the concentrations of the salts used, the ordinates represent the rate of respiration.

Fig. 3 shows antagonism between NaCl and Na taurocholate. In this figure the abscissæ represent the molecular proportions of each component in the mixture: thus the ordinate at the extreme left represents the rate in a pure solution of NaCl; the ordinate at the extreme right, the rate in a solution containing 95 parts of NaCl and 5 parts of Na taurocholate. In a solution containing 1 part of Na taurocholate

FIG. 3. Curve showing antagonism between NaCl and Na taurocholate. The ordinates represent the rate of respiration (expressed as per cent of the normal); the abscissæ represent molecular proportions of the solution used. Thus the ordinate at the extreme left represents the rate in 0.8 M NaCl while the ordinate at the extreme right (in the lower figure) represents the rate in 95 parts of NaCl and 5 parts of Na taurocholate (molecular proportions). Mixtures in which the proportion of Na taurocholate was greater showed no respiration.

Since the changes near the beginning of the curve could not be shown accurately this portion is represented on an enlarged scale in the upper part of the figure. The optimum proportion for the production of CO₂ is 99.9931 parts of NaCl and 0.0069 parts of Na taurocholate. Normal rate as in Fig. 1. Average of three experiments; probable error of the mean less than 2 per cent of the mean.

to 14,375 parts of NaCl the rate of respiration was practically normal. In all other proportions it was less than normal. For the sake of clearness, the portion of the figure at the extreme left has been drawn on an enlarged scale and is inserted above. These proportions are quite different from those found by Osterhout (500 of NaCl to 1 of Na taurocholate) to be most favorable, but the difference may be due to the

fact that in the experiments reported by Osterhout the highest proportion of NaCl used was 500 to 1. Since this shows good antagonism, higher proportions were not tried, as the object of the experiment was merely to demonstrate antagonism and not to ascertain the most favorable mixture.

Owing to the unusual results obtained with high dilutions of Na taurocholate, it was thought advisable to procure Na taurocholate from several different sources. The original product used was Merck's. An additional sample was obtained from Eimer and Amend, and some purified bile salts were kindly sent by Dr. Morgulis and Dr. Hecht. Experiments made with all the above different samples were in remarkably good agreement, thus showing that the results were not due to the impurities which might have been present in the Na taurocholate.

Experiments on recovery were made with a mixture of NaCl and Na taurocholate (which produced a decrease in the rate of respiration). In the course of an hour, after removal of the bacteria from the mixture to 0.75 per cent dextrose, the rate did not quite attain the normal.

A few experiments with saponin were tried, inasmuch as it seemed possible that the same mechanism might be responsible for the action of Na taurocholate and for that of saponin. Eighteen concentrations of saponin were used (ranging from 0.001 to 0.0000005 M), including those which produce an increase in the rate of respiration in the case of Na taurocholate. It was found that the concentrations which were greater than 0.00005 M produced a progressive decrease in the rate, while those which were less than 0.00005 M gave the normal rate of respiration (100 per cent). None gave an increase in the rate of respiration.

Both saponin and Na taurocholate are exceedingly effective in lowering surface tension but their effects on respiration are so different as to indicate that surface tension does not play an important part in this connection. This conclusion would not, however, be justified if it could be shown that saponin is unable to penetrate the cell. The fact that in the highest concentrations employed⁶ there was some

⁶ Higher concentrations were not employed on account of the foaming which they produced.

depression⁷ of the rate of respiration indicates that saponin penetrates to some extent.

It is interesting to observe that the results obtained with *Bacillus subtilis* resemble those obtained by Osterhout in measuring the electrical conductivity of *Laminaria*, since in both cases the effect of saponin⁸ is very much less than that of Na taurocholate.

SUMMARY.

1. The addition of Na taurocholate produces an increase in the rate of respiration at a concentration of 0.0000125 M, and a decrease at 0.001 M and in higher concentrations.

2. NaCl is antagonized by Na taurocholate, the most favorable proportion being 14,375 parts of NaCl to 1 part of Na taurocholate (molecular proportions).

3. Solutions of saponin, at concentrations from 0.00005 M to 0.001 M, decrease the rate of respiration: lower concentrations produce no effect.

⁷ The depression was not due to the buffer action of the saponin. This was shown by comparing the change in pH produced in the saponin solution and in distilled water when a solution of carbonic acid was added to each.

⁸ Unpublished results of experiments on *Laminaria*.